Scientific Opinion on erucic acid in feed and food

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Erucic acid in feed and food

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Abstract

Erucic acid is the trivial name of the fatty acid *cis*-13-docosenoic acid and occurs at high concentrations mainly in the seeds of species of the Brassicaceae (e.g. rape seed or mustard seed). The European Commission requested EFSA to deliver a scientific opinion on the risks for animal and human health related to the presence of erucic acid in feed and food. For most humans, the main contributor to dietary exposure to erucic acid was the food group 'Fine bakery wares'. In 'Infants', 'Food for infants and small children' was the main contributor to exposure. The heart is the principal target organ for toxic effects after exposure. Myocardial lipidosis was identified as the critical effect for chronic exposure to erucic acid. This effect is reversible and transient during prolonged exposure. A tolerable daily intake (TDI) of 7 mg/kg body weight (bw) per day for erucic acid was established, based on a no observed adverse effect level of 0.7 g/kg bw per day for lipidosis in young rats and newborn piglets. Mean chronic exposure of the different groups of the population did not exceed the TDI. The two highest 95th percentile dietary exposure levels were observed for infants (ranging from 1.7 to 7.4 mg/kg bw per day, minimum lower bound (LB) – maximum upper bound (UB)) and other children (ranging from 2.1 to 9.5 mg/kg bw per day, minimum LB – maximum UB), the last max UB estimate being above the TDI. This may indicate a risk for young individuals with high erucic acid exposure. In pigs, levels of erucic acid are unlikely to represent a health concern. However, for poultry, the small margin between the lowest observed adverse effect level (LOAEL) and the estimated exposure may indicate a health risk where maximum inclusion rates are applied. Due to the absence of adequate data, the risk for ruminants, horses, fish and rabbits could not be assessed.

Keywords: erucic acid, rape seed, rapeseed oil, risk assessment, food, feed, *cis*-13-docosenoic acid

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Erratum: Following an official communication in January 2017 from one data provider on the submission of incorrect occurrence data for several samples of rapeseed oil, the Scientific Opinion on erucic acid in feed and food adopted by the CONTAM Panel on 21 September 2016 was revised. In particular, human dietary exposure was re-assessed using the corrected occurrence data. The following sections of the Scientific Opinion were corrected: abstract, summary, current occurrence data, dietary exposure assessment of erucic acid in humans, human health risk characterisation, conclusions and appendix C. The amended document was readopted by the CONTAM Panel on 5 April 2017. To avoid confusion, the older version has been removed from the EFSA Journal, but is available on request, as is a version showing all the changes made.

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Summary

Following a request from the European Commission, the EFSA Panel on Contaminants in the Food Chain (CONTAM Panel) was asked to deliver a scientific opinion on the risks for animal and human health related to the presence of erucic acid in feed and food. The Scientific Opinion should, inter alia, comprise the (a) evaluation of the toxicity of erucic acid for animals and humans, considering all relevant adverse health effects; (b) estimation of the dietary exposure of the European Union (EU) population to erucic acid including the consumption patterns of specific (vulnerable) groups of the population (e.g. high consumers, children, people following a specific diet, etc.); (c) estimation of the exposure of the different animal species to erucic acid in feed and the level of transfer/carryover of erucic acid from the feed to the products of animal origin for human consumption resulting in unacceptable levels of erucic acid; (d) assessment of the human health risks for the EU population including for specific (vulnerable) groups of the population as the consequence of the estimated dietary exposure; (e) assessment of the animal health risks for the different animal species as the consequence of the estimated exposure from animal feed.

Erucic acid is the trivial name of the fatty acid *Z*-13-docosenoic or *cis*-13-docosenoic acid. According to the most common nomenclature for fatty acids, erucic acid is abbreviated as 22:1 n-9 or 22:1 ω-9.

Erucic acid is present at high concentrations mainly in the seeds of species of the Brassicaceae (e.g. rape seed or mustard seed and also seeds from vegetable crops such as kales, cabbages and turnips). Although natural forms of rapeseed and mustard species contain high levels of erucic acid, usually more than 40% of the total fatty acids, commercially bred cultivars of rapeseed developed since the 1970s have low levels of erucic acid, typically less than 0.5%. High erucic acid rapeseed (HEAR) cultivars are still grown to meet the demand of the oleochemical industry but are exclusively intended for non-food uses. Mustard seed production is based on cultivars with high erucic acid content. Erucic acid is also present at low concentrations in other food sources such as fish.

Following an ad-hoc data collection, a final dataset of 12,444 food samples representing most of the food commodities with potential presence of erucic acid was available to estimate dietary exposure. Samples were collected between 2000 and 2015 (half of them in 2014) in 15 different European countries, however, most of them being from one Member State. The percentage of left-censored data reported (results below limit of detection and/or limit of quantification) was high (69%). The highest number of reported samples corresponded to the food group 'Animal and vegetable fats and oils' (~ 60%) and in particular to 'Rapeseed oil' (n = 5,832). Other food groups that were well represented were 'Starchy roots and tubers' (n = 1,223), 'Grains and grain-based products' (n = 982) and 'Food for infants and small children' (n = 810).

Mean values reported in rapeseed oil were 1,285/5,215 mg/kg (lower bound (LB)/upper bound (UB)) with about 80% being left-censored data. The presence of erucic acid in 'Fine bakery wares' indicates the common use of rapeseed oil in the preparation of these products. For 'Pastries and cakes', erucic acid was quantified in half of the samples (mean 240/290 mg/kg (LB/UB)) and for 'Biscuits' in about 25% of the samples (mean 270/390 mg/kg (LB/UB)). Overall, relatively low levels of erucic acid were reported for the food group 'Food for infants and small children'. The highest mean values were reported for 'Infant formulae, powder' (220/290 mg/kg (LB/UB)) and the lowest for 'Ready-to-eat meal for infants and young children' (77/86 mg/kg (LB/UB)). The latest version of the EFSA Comprehensive European Food Consumption database was used and consumption data were classified according to the FoodEx classification system. Based on the critical effect identified in toxicity studies, the CONTAM Panel considered that only chronic dietary exposure to erucic acid had to be assessed. The highest chronic dietary exposure was estimated in the youngest population. For the mean dietary exposure, the highest estimate at the LB corresponded to the age classes 'Infants' and 'Toddlers' with a maximum value of 2.8 mg/kg body weight (bw) per day, while at the UB the maximum estimate was observed in the age class 'Toddlers' (4.4 mg/kg bw per day). In the highly exposed population (95th percentile), the highest estimates were in 'Infants' (5.8/7.4 mg/kg bw per day (LB/UB)) and 'Other Children' (5.3/9.5 mg/kg bw per day (LB/UB)).

Overall, the food group 'Fine bakery wares', more precisely 'Pastries and cakes' and 'Biscuits (cookies)' was the main contributor of dietary exposure to erucic acid. At the middle bound (MB), the contribution of 'Fine bakery wares' in 'Toddlers' represented up to 39% of the total exposure (median = 21%) and in 'Other children' contributed up to 48% to the total exposure (median = 27%). Since the levels of erucic acid in 'Fine bakery wares' ('Pastries and cakes' and 'Biscuits (cookies)') were not that high (240/390 mg/kg (LB/UB)), its relevant contribution is mainly driven by the high consumption of this heterogeneous food category (e.g. croissants, doughnuts, cakes, muffins, waffles,

biscuits, cookies, etc.). In the age class 'Infants', 'Food for infants and small children' (FoodEx level 1) was the main contributor to the exposure. The contribution of the food group 'Ready-to-eat meal for infants and young children' reached 52% at the MB scenario (range 19–52%) among the dietary surveys for 'Infants'.

The contribution of rapeseed oil to the total dietary exposure to erucic acid was, in most of the cases, limited. However, in few dietary surveys, the consumption of rapeseed oil played an important role reaching average contributions (MB scenario) up to 63%, with an average contribution of 39% in the dietary survey with the highest exposure estimate.

Specific exposure scenarios (consumers only) were used to estimate the potential exposure via the consumption of 'Composite foods' and 'Custard'. Both maximum mean exposure (UB) and maximum 95th percentile dietary exposure (UB) via the consumption of prepared pasta alone were around 6-fold higher than the maximum exposure estimates in 'Toddlers' and 'Adults' considering the whole diet.

Only 275 feed samples were available to estimate animal dietary exposure to erucic acid, most of the samples collected in the EU between 2003 and 2015. Most of the feed samples referred to 'Rapeseed oil' (n = 193). The erucic acid content of only 28 samples of rapeseed expeller was provided, and no data for rapeseed meal, which is the more commonly used feed. The highest average levels of erucic acid were reported for 'Rapeseed oil' (1,300/4,200 mg/kg (LB/UB)).

Rapeseed cakes, meals and oils are important feed materials in diets for livestock in the EU. Exposures to erucic acid by farmed livestock were estimated using typical feed intakes and body weights, and feed industry guidelines for the maximum inclusion rates of rapeseed meal and oil in livestock diets. The estimated mean exposures therefore represent worst-case scenarios. Insufficient data on levels of erucic acid in rapeseed meal or cake were available to allow P95 estimates of exposure to be made. Since rapeseed oil or meals are not commonly included in diets for cats and dogs, no estimates of exposure have been made for these animals. In the category 'Ruminants and horses', the highest exposure was for lactating goats (5.0/7.5 mg/kg bw per day (LB/UB)). For pigs and poultry, the highest exposures were for fattening chickens (9.4/12 mg/kg bw per day (LB/UB)).

Erucic acid is present in food and feed, predominantly as component of triacylglycerols. It is well absorbed from the gastrointestinal tract to an extent varying between 60% and 100%, depending on the species. Humans exhibit virtually complete absorption. Erucic acid is distributed to all organs; however, there is little distribution into the brain. Mitochondrial β-oxidation of erucic acid is poor in rats and pigs. Human heart mitochondria appear to also have low activity for erucic acid. Little is known regarding the excretion of erucic acid.

There is evidence that erucic acid in the feed is transferred to products of animal origin and a dose-related increase in erucic acid in food of animal origin has been shown. In ruminants, erucic acid is also partially hydrogenated or isomerised in the rumen.

The heart is the principal target organ for toxic effects following short-term or long-term exposure of rats, pigs, monkeys, rabbits and gerbils to diets with oils containing erucic acid. The most common and sensitive effect observed in all species is myocardial lipidosis, i.e. an accumulation of triacylglycerols in myocardium that appear as neutral lipid droplets. Lipidosis is reversible and transient during prolonged exposure. Studies in rats and pigs showed an association between the level of erucic acid in the diet and the severity of myocardial lipidosis. In rats, increased myocardial lipidosis is observed at doses of 1 g erucic acid/kg bw per day or higher. In newborn pigs, increased myocardial lipidosis is observed at a dose of 1.1 g erucic acid/kg bw per day. The overall no observed adverse effect level (NOAEL) for lipidosis was 0.7 g/kg bw per day in a 7-day feeding study in young rats and in a 2-week feeding study in newborn piglets. Adult pigs are able to tolerate higher levels of erucic acid than young animals. These results suggest that the immature myocardium and/or liver may be less able to metabolise erucic acid, making neonates especially prone to myocardial lipidosis, albeit transiently. Myocardial lipidosis is reported to reduce the contractile force of the heart muscle. Mitochondrial damage (megamitochondria, mitochondrial proliferation, increase in the average volume, distortion of shape, degeneration) and disorganisation of myofibrils has been reported after exposure of rats, pigs, monkeys or rabbits to high doses of erucic acid.

Feeding rats with high erucic acid doses for 4 or more weeks is associated with the occurrence of myocardial necrosis and fibrosis. Factors other than, or in addition to, erucic acid are likely to be responsible for the increased incidence of these lesions, e.g. fatty acid imbalance. Therefore, the CONTAM Panel considered necrosis not a suitable endpoint for the risk assessment. A causal link between myocardial lipidosis and myocardial lesions has not been established.

Non-cardiac effects, such as changes in the liver, kidneys, skeletal muscle, adrenals and testis weight, have also been reported in rats and haematological and liver alterations in newborn piglets. In

all cases, these effects are observed at somewhat higher doses than those leading to cardiac lipidosis in the same species.

Because of the lack of adequate studies, no conclusions can be drawn on the genotoxicity and carcinogenicity of erucic acid. No major adverse reproductive and developmental effects were associated with feeding female rats, mice and hamsters with erucic acid-containing diets prior to mating and during pregnancy.

In humans, a higher level of 22:1 in plasma phospholipids has been associated with higher incidence of congestive heart failure in two independent cohorts whereas higher circulating levels of erucic acid in erythrocytes have been associated with lower incidence of coronary heart disease. Two studies on the possible association between cancer and erucic acid exposure were identified but no conclusion can be drawn due to the intrinsic limitations or lack of specificity of the outcome. The therapeutic use of erucic acid, as component of Lorenzo's oil for treating adrenoleukodystrophy (ALD) patients, results in haematological effects, most notably thrombocytopenia and morphological alterations of thrombocytes, at doses of about 0.1 g/kg bw per day. Erucic acid induced lipidosis has not been described in humans.

A high intake of erucic acid leads to lipidosis in pigs and rats, particularly in the heart, due to the poor β-oxidation of erucic acid in mitochondria. Cardiac lipidosis is transient (reversible), even after prolonged intake of erucic acid because of the induction of peroxisomal degradation of erucic acid.

A reduction in feed intake and milk yield by dairy cows was reported at an intake of 0.4 g erucic acid/kg bw per day from rapeseed meal. However, the possible role of glucosinolates or other antinutritional factors in the meal could not be ruled out. Feeding poultry with diets containing HEAR oil resulted in growth retardation and cardiac lipidosis. High doses of erucic acid also increased the incidence and severity of cardiac lesions (similar to those observed in the rat). In addition, hydropericardium, effects on the liver and skeletal muscles were induced in several species fed diets containing high doses of erucic acid. The CONTAM Panel identified a lowest observed adverse effect level (LOAEL) of 0.02 g/kg bw per day for liver toxicity in poultry. Studies, in which poultry were fed diets supplemented with oils and meals derived from HEAR cultivars, clearly demonstrated adverse effects on production-related factors. However, as for other livestock, the possible effects of other dietary constituents or characteristics on feed intake, growth rate and egg production cannot be excluded. No conclusion regarding the adverse effects in fish, rabbits and horses could be drawn due to the limited studies available. No studies on adverse effects in companion animals were identified.

An acute reference dose was not established because of the lack of endpoints indicative of acute toxicity on target organs. Myocardial lipidosis, as reported in rats and pigs following feeding with HEAR oils, was selected as critical effect for establishing a tolerable daily intake (TDI) for erucic acid. Since there are no adequate data from human studies for dose–response assessment, the CONTAM Panel considered the data from studies on experimental animals to identify reference points. The CONTAM Panel selected the overall NOAEL for lipidosis of 0.7 g/kg bw per day, observed in a 7-day feeding study in young rats and in a 2-week feeding study in newborn piglets, as reference point for the risk assessment. Based on this NOAEL, the CONTAM Panel established a TDI of 7 mg/kg bw for erucic acid using the default uncertainty factor of 100 to account for intra- and interspecies differences. The CONTAM Panel noted that this TDI is well below the erucic acid dose of 100 mg/kg bw per day causing haematological effects in ALD patients treated with Lorenzo's oil.

Data on human dietary exposure to erucic acid across dietary surveys and age groups showed mean exposure values that ranged from 0.3 (minimum LB) to 4.4 mg/kg bw per day (maximum UB) across dietary surveys and age groups. The mean dietary exposure for adults ranged from 0.3 to 1.9 mg/kg bw per day across the European surveys, and it was the highest for toddlers, ranging from 1.2 to 4.4 mg/kg bw per day, which is below the TDI of 7 mg/kg bw per day. The 95th percentile dietary exposure levels ranged from 0.7 (minimum LB) to 9.5 mg/kg bw per day (maximum UB) across dietary surveys and age groups, with a range for adults from 0.9 to 4.3 mg/kg bw per day. The 95th percentile dietary exposure level was highest in infants (ranging from 1.7 to 7.4 mg/kg bw per day, minimum LB – maximum UB) and other children (ranging from 2.1 to 9.5 mg/kg bw per day, minimum LB – maximum UB), the last maximum UB estimate being above the TDI. This may indicate a risk for young individuals with high erucic acid exposure. However, it should be noted that only one exposure estimate (UB) was above the TDI across all the different dietary surveys.

For ruminants, no NOAEL could be identified. The dietary exposure of dairy cattle is well below the dose at which no effect is observed on milk yield. However, the risk of other adverse effects or for other ruminants could not be assessed. For pigs, the CONTAM Panel identified a NOAEL of 700 mg/kg bw per day for myocardial lipidosis. The dietary exposure of pigs is well below the NOAEL for lipidosis

in pigs. For poultry, a LOAEL of 20 mg/kg bw per day was identified for liver toxicity, which is about double the upper exposure range (12 mg/kg bw per day). The small margin between the LOAEL and the estimated exposure may indicate a health risk for poultry where maximum inclusion rates are applied. It should be mentioned that insufficient data on levels of erucic acid in rapeseed meal or cake were available to allow P95 estimates of exposure to be made. No NOAEL/LOAEL could be identified for horses, fish and rabbits, and therefore, the risk of erucic acid exposure could not be assessed for these species. However, the CONTAM Panel noted that the exposure of horses (0.95/1.5 mg/kg bw per day (LB/UB)) and rabbits (6.4/13 mg/kg bw per day (LB/UB)) is well below the NOAEL of 700 mg/kg bw per day for pigs. The dietary exposure of cats and dogs to erucic acid is considered to be negligible and no risk characterisation has been undertaken.

Several uncertainties concerning the exposure assessment and hazard identification and characterisation have been identified. The CONTAM Panel considered that the impact of the uncertainties on the risk assessment of human and animal exposure to erucic acid through consumption of food and feed is considerable. Based on the lipidosis selected as the critical effect to derive the TDI, the CONTAM Panel concluded that the risk assessment of human exposure to erucic acid presented in the opinion is more likely to overestimate than to underestimate the risk. For the same reason, the CONTAM Panel concluded that the risk assessment for pigs is more likely to overestimate than to underestimate the risk. The use of maximum inclusion rates resulted in an overestimation of the exposure for poultry. However, considering the uncertainties related to the use of a LOAEL as toxicological reference point for poultry, it is not possible to conclude whether the risk for this species is overestimated.

The EFSA CONTAM Panel recommends the generation of more analytical data on the occurrence of erucic acid in relevant food and feed commodities using sensitive and specific methods. Special attention should be paid to processed foods such as 'Fine bakery wares', 'Food for infants and small children' and 'Composite foods'. There should be more information on the levels in animal-derived products (meat, milk and eggs) resulting from the transfer of erucic acid from animal feed. There is a need for a repeated-dose toxicity study in newborn rats or pigs with pure erucic acid in order to clarify the potential confounding effects of other fatty acids present in the oil and to provide information regarding the dose–response relationship. Moreover, studies should be conducted on species differences in the cardiac and hepatic metabolism of erucic acid. Further studies are also required to determine reference points for target livestock animals and fish.

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1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

Background

A maximum level for erucic acid in oils and fats intended as such for human consumption and in foodstuffs containing added oils and fats has been established by Council Directive 76/621/EEC[1]. A stricter maximum level for erucic acid has been established in infant formulae and follow-on formulae by Commission Directive 2006/141/EC.[2]

Erucic acid is a natural plant toxin which is a contaminant according to the definition of the contaminant provided in Council Regulation (EEC) No 315/93 of 8 February 1993 laying down Community procedures for contaminants in food[3] as the presence of erucic acid in food is the result of the agricultural production, more in particular the choice of the variety.

To simplify the legislation, the maximum levels for erucic acid have been established in Regulation (EC) 1881/2006[4], as amended by Commission Regulation (EU) No 696/2014[5]. Council Directive 76/621/EEC shall be repealed subsequently.

The appropriateness of setting a maximum level for erucic acid has been highlighted by the Scientific Committee on Food (SCF) in its opinion expressed on 17 September 1993 on essential requirements for infant formulae and follow-on formulae.[6]

When the maximum levels of erucic acid were established by Regulation (EC) 1881/2006, it was found necessary to review the maximum levels in the future based upon an updated risk assessment and also to consider the appropriateness of establishing maximum levels for erucic acid in feed.

Terms of Reference

In accordance with Art. 29 (1) (a) of Regulation (EC) No 178/2002, the Commission asks the European Food Safety Authority (EFSA) for a scientific opinion on the risks for animal and human health related to the presence of erucic acid in feed and food.

The scientific opinion should, inter alia, comprise the:

a) evaluation of the toxicity of erucic acid for animals and humans, considering all relevant adverse health effects;
b) estimation of the dietary exposure of the European Union (EU) population to erucic acid including the consumption patterns of specific (vulnerable) groups of the population (e.g. high consumers, children, people following a specific diet, etc.);
c) estimation of the exposure of the different animal species to erucic acid in feed and the level of transfer/carryover of erucic acid from the feed to the products of animal origin for human consumption resulting in unacceptable levels of erucic acid;
d) assessment of the human health risks for the EU population including for specific (vulnerable) groups of the population as the consequence of the estimated dietary exposure;
e) assessment of the animal health risks for the different animal species as the consequence of the estimated exposure from animal feed.

1.2. Interpretation of the Terms of Reference

The EFSA Panel on Contaminants in the Food Chain (CONTAM) concluded that the terms of reference provided by the Commission were clear.

[1] Council Directive 76/621/EEC of 20 July 1976 relating to the fixing of the maximum level of erucic acid in oils and fats intended as such for human consumption and in foodstuffs containing added oils and fats. OJ L 202, 28.2.1976, p. 35–37.
[2] Commission Directive 2006/141/EC of 22 December 2006 on infant formulae and follow-on formulae and amending Directive 1999/21/EC. OJ L 401, 30.12.2006, p. 1–33.
[3] Council Regulation (EEC) No 315/93 of 8 February 1993 laying down Community procedures for contaminants in food. OJ L 37, 13.2.1993, p. 1–3.
[4] Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. OJ L 364, 20.12.2006, p. 5–24.
[5] Commission Regulation (EU) No 696/2014 of 24 June 2014 amending Regulation (EC) No 1881/2006 as regards maximum levels of erucic acid in vegetable oils and fats and foods containing vegetable oils and fats. OJ L 184, 25.6.2014, p. 1–2.
[6] http://ec.europa.eu/food/fs/sc/scf/reports/scf_reports_34.pdf

1.3. Additional information

1.3.1. Oil and oil seeds containing erucic acid

Erucic acid is present at high concentrations mainly in the seeds of some species of the plant family Brassicaceae. Around 80–90% of the species of this family naturally contain varying amounts of erucic acid that account for up to 60% of the total fatty acids (Kumar and Tsunoda, 1980; Goffman et al., 1999; Velasco et al., 1999). This family contains several species that are cultivated for their seeds, mainly for seed oil production but also for manufacturing condiments such as mustard. Most of them belong to the genus *Brassica*, including rapeseed (*Brassica napus* L.), turnip rape (*Brassica rapa* L.), Indian or oriental mustard (*Brassica juncea* [L.] Czern.), Ethiopian or Abyssinian mustard (*Brassica carinata* A. Braun.) and black mustard (*Brassica nigra* [L.] Koch) (Velasco and Fernández-Martínez, 2009). *Brassica rapa* is commonly found in the literature as *Brassica campestris*, although the former name is preferred according to the International Code of Botanical Nomenclature (Dixon, 2006). Therefore, *B. rapa* is used in this opinion with precedence over *B. campestris*. The CONTAM Panel noted that *B. rapa* is also mistakenly referred to as rapeseed by some authors. Other species cultivated for their seeds are white mustard (*Sinapis alba* L.), crambe (*Crambe hispanica* subsp. *abyssinica* [Hochst. ex R. E. Fr.] Prina), camelina (*Camelina sativa* [L.] Crantz) and eruca (*Eruca vesicaria* subsp. sativa [Mill.] Thell.) (Warwick, 2011). Most of these species are also cultivated as vegetable and/or fodder crops (Velasco and Fernández-Martínez, 2009; Warwick, 2011). Another species, radish (*Raphanus* spp.), contains forms cultivated for their edible young seed pods and as animal fodder (Warwick, 2011). Additionally, seeds from some Brassicaceae species, including some of the abovementioned species plus others such as *Brassica oleracea* L., have become popular to produce sprouts for salads (West et al., 2002), whereas a diversity of plant parts are used to make pickles (Ayaz et al., 2006).

When in this Scientific Opinion the erucic acid content is reported as a percentage, this value refers to the percentage erucic acid in the total fatty acids on a weight basis.

The most commercially important crop of the Brassicaceae is rapeseed, which is produced at a higher scale compared to turnip rape (Booth and Gunstone, 2004). Both crops are nowadays cultivated worldwide over a global acreage close to 34 million hectares, yielding a production of 23 million tonnes seed oil. Around 20% of world acreage of rapeseed and turnip rape, which yields 38% of the world rapeseed oil production, is located in the EU.[7] Turnip rape has a more restricted distribution area than rapeseed including parts of Sweden and Finland, western Canada, north-western China, and the Indian subcontinent (Velasco and Fernández-Martínez, 2009).

Although natural forms of rapeseed, turnip rape and mustard species contain high levels of erucic acid, usually more than 40%, commercially bred cultivars of rapeseed and other species developed since the 1970s have been essentially free from erucic acid, i.e. < 1% (Friedt and Snowdon, 2009). In Canada, the term 'canola' was created to refer to cultivars with low erucic acid content and low glucosinolate content. The official definition of canola is as follows:[8]

> 'Seeds of the genus *Brassica* (*Brassica napus*, *Brassica rapa* or *Brassica juncea*) from which the oil shall contain less than 2% erucic acid in its fatty acid profile and the solid component shall contain less than 30 micromoles of any one or any mixture of 3-butenyl glucosinolate, 4-pentenyl glucosinolate, 2-hydroxy-3-butenyl glucosinolate and 2-hydroxy-4-pentenyl glucosinolate per gram of air-dry, oil-free solid'.

Today the term canola is used worldwide, including in scientific literature, to refer to low erucic acid and low glucosinolate content cultivars of *Brassica* species. Current rapeseed varieties are characterised by extremely low erucic acid content, usually below 0.5% (Temple-Heald, 2004). Evaluation of erucic acid content in the rapeseed cultivars marketed in France from 2005 to 2015 resulted in all cases in average erucic acid values below 0.5% (Appendix A, Table A.1). The Canadian Grain Commission reported an average erucic acid content of 0.01% in rapeseed and rapeseed oil samples collected from producers, crushing plants and grain handling offices across Western Canada from 2009 to 2014 (Barthet, 2014). Comparative fatty acid composition of high erucic acid rapeseed (HEAR) and low erucic acid rapeseed (LEAR) cultivars is shown in Table 1.

[7] http://faostat3.fao.org/home/index.html
[8] http://www.canolacouncil.org/oil-and-meal/what-is-canola/

Table 1: Comparison of the average percentage of major fatty acids in the seed oil of high erucic acid rapeseed (HEAR) and low erucic acid rapeseed (LEAR) cultivars (Gunstone and Harwood, 2007)

Fatty acid	LEAR	HEAR
Palmitic acid 16:0	3.6	4.0
Stearic acid 18:0	1.5	1.0
Oleic acid 18:1 n-9	61.6	14.8
Linoleic acid 18:2 n-6	21.7	14.1
Linolenic acid 18:3 n-3	9.6	9.1
Gondoic acid 20:1 n-9	1.4	10.0
Erucic acid 22:1 n-9	0.2	45.1

HEAR cultivars are still grown to meet the demand of the oleochemical industry. Currently, world demand for high erucic acid oil is estimated to be around 0.1 million tonnes (Zanetti et al., 2012). In the EU, high erucic acid oil production is mainly located in the United Kingdom (UK), where high erucic acid cultivars are grown under contract at a minimum distance of 50 m from low erucic rapeseed crops to avoid cross-pollination between both types of cultivars (Temple-Heald, 2004). Acreage of HEAR in the UK is estimated in more than 25,000 ha.[9] There is also a small-scale production of high erucic varieties in Germany and France, estimated to be 20,000 and 14,000 ha, respectively (Merrien, 2011). It is also produced in several countries outside the EU such as China and India.

Crambe oil is one of the oils with the highest erucic acid content, typically over 60% (Lalas et al., 2012). It is exclusively intended for non-food uses (Warwick, 2011). Camelina oil has a relatively low erucic acid content that typically ranges from 1% to 4%. Currently, camelina is a very minor crop that is perceived by breeders as a potential crop for both food and non-food uses, the latter particularly in the field of biofuels (Vollmann and Eynck, 2015).

Oils from white mustard, black mustard and eruca are mainly produced in India at a small scale (Grubben and Denton, 2004; Warwick, 2011; Shekhawat et al., 2012). Seed oils from these three species contain high erucic acid levels over 30% (Goffman et al., 1999). Forms with low erucic acid content have been developed for white mustard (Raney et al., 1995), but there is no indication that seed oil production in India is based on low erucic acid cultivars.

Erucic acid is also present in the seeds and seed oils of other plant families, usually at lower concentrations than in the Brassicaceae family. For example, meadow foam (*Limnanthes alba* Hartw. ex Benth.; Limnanthaceae) is a North American minor annual crop producing a seed oil of great value for the cosmetic industry. It naturally contains between 8% and 24% of erucic acid, although improved forms with lower erucic acid levels (< 3%) have been developed (Gandhi et al., 2009). Borage (*Borago officinalis* L.; Boraginaceae) seeds produce an oil marketed as a dietary supplement on the basis of alleged nutritional benefits associated with the presence of γ-linolenic acid (Ziboh, 2008). Borage oil typically contains between 1% and 3% erucic acid, although forms with higher erucic acid content have been identified in the wild (de Haro et al., 2002). Erucic acid levels below 3% are also found in the seeds of some lupine species such as white lupine (*Lupinus albus* L.), consumed as a popular snack in southern Europe and also used for animal feeding (Bhardwaj et al., 2004), although higher values, up to 5%, have been reported in some environments (Boschin et al., 2008). Erucic acid can also be found at low levels in the seed lipids of other species such as cowpea (*Vigna unguiculata* [L.] Walp.) (Antova et al., 2014) and quinoa (*Chenopodium quinoa* Willd.) (Wood et al., 1993).

Seeds from several species of the Brassicaceae family are used to produce mustard. Annual world production of mustard seed not intended for oil extraction is around 0.7 million tonnes, with Canada and Nepal being the two major producers.[7] In Canada, three types of mustard are grown: yellow mustard (*S. alba*), and oriental and brown mustard, which are two groups of varieties of *B. juncea*, differing mainly in seed colour.[10] These three types contain high levels of erucic acid: around 25% in brown and oriental mustard and around 35% in yellow mustard (Siemens, 2014). In the EU, Germany is the main producing country of mustard seed, with a production of around 10,000 tonnes/year.

Several species of the Brassicaceae family comprise highly consumed vegetables. The most important group is *B. oleracea*, which encompasses a great diversity of vegetable forms such as

[9] http://www.premiumcrops.com/files/Agronomist_Briefing_HEAR.pdf
[10] http://saskmustard.com/grower/manual/plant-description/types-of-mustards-and-their-uses/index.html

cabbage, broccoli, cauliflower, kale, Brussels sprouts, collard greens and kohlrabi. *Brassica* vegetables may contain only traces of erucic acid, while the seeds contain high levels. For example, mature kale leaves have been reported to have an erucic acid content of 2 mg/kg (dry weight basis) (Ayaz et al., 2006), and a very small amount of erucic acid has been detected in broccoli florets, around 8 mg/kg (fresh weight basis), compared to 120,500 mg/kg in broccoli seeds (West et al., 2002). High levels of erucic acid of up to 87,280 mg/kg have also been reported in sprouts from *Brassica* seeds (Bhardwaj and Hamama, 2009).

The Brassicaceae family also encompasses important fodder crops such as forage rape (*B. napus*), turnips (*B. rapa*), kales and cabbages (*B. rapa* and *B. oleracea*), swedes (*B. napus*) and fodder radishes (*Raphanus sativus*). Erucic acid is only present in the seeds of these Brassicaceae crops (Peiretti et al., 2012) and since these crops are grazed in vegetative stages of development, they are not a source of erucic acid.

Besides the occurrence in oil seeds, erucic acid also occurs in fish and marine animals and human milk (see Section 3.1.1.1).

1.3.2. Previous assessments

In 2003, the Food Standards Australia and New Zeeland (FSANZ) published a review of the health effects of erucic acid (FSANZ, 2003). In the absence of adequate human data, a no observed effect level (NOEL) of 750 mg/kg body weight (bw) per day, based on the occurrence of increased myocardial lipidosis at 900 mg/kg bw per day in newborn pigs (Kramer et al., 1990), was considered appropriate for extrapolation to humans. By applying an uncertainty factor of 100 (10 for extrapolation from experimental animals to humans, 10 for variation within humans), a tolerable level for human exposure of 7.5 mg erucic acid/kg bw per day (about 500 mg erucic acid/day for the average adult) was calculated and proposed as the provisional tolerable daily intake (pTDI) for erucic acid. Moreover, it was concluded that, with the current mean dietary intakes of erucic acid in canola oil by Australian and New Zealand consumers, there is no cause for concern in terms of public health and safety, but high consumers of canola oil have the potential to approach the pTDI.

In 1995, the SCF (1995) recommended that the content of erucic acid in infant formulae should not exceed 1% of total fatty acids. This precautionary decision was based on toxicological studies showing higher severity of myocardial lipidosis induced by erucic acid in newborn piglets when compared to weaned pigs (Kramer et al., 1990) and on the observation that erucic acid may inhibit fatty acid elongation in human cells (Koike et al., 1991).

In 2003, the SCF recommended to maintain a maximum level of erucic acid of 1% total fatty acids in infant formulae and follow-on formulae as indicated in the Infant Formulae Directive[11] (SCF, 2003).

1.3.3. Chemistry

Erucic acid is the trivial name used for the fatty acid with systematic name *Z*-13-docosenoic or *cis*-13-docosenoic acid according to International Union of Pure and Applied Chemistry (IUPAC) nomenclature. This can be abbreviated as 22:1Δ13*c* or 22:1 13*c* (Scrimgeour and Harwood, 2007; Lobb and Chow, 2008). This nomenclature denotes a molecule with 22 carbon atoms and one double bond in *cis* configuration located between the carbon atoms at positions 13 and 14 counting from the carboxylic group (Figure 1).

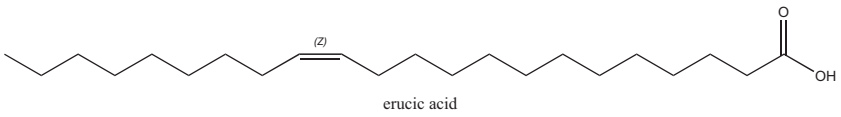


Figure 1: Molecule of *Z*-13-docosenoic acid (erucic acid)

Notwithstanding this, the most common nomenclature for fatty acids in scientific literature counts the position of the first carbon of double bonds starting from the methyl group of the fatty acid and denotes the position of the first double bond with the prefixes n- or ω-. Double bonds are assumed to be in *cis* geometric configuration unless otherwise indicated. With this nomenclature, erucic acid is abbreviated as 22:1 n-9 or 22:1 ω-9 (Lobb and Chow, 2008). The presence of *trans* configuration is indicated in this Scientific Opinion as *trans* 22:1 n-9, which is brassidic acid.

[11] Commission Directive 91/321/EEC of 14 May 1991 on infant formulae and follow-on formulae. OJ L 175, 4.7.1991, p. 35–49.

The main identifiers[12] as well as physical and chemical properties of erucic acid are summarised in Table 2.

Table 2: Main identifiers and physical and chemical properties of erucic acid

Identifier/property	Value
CAS Registry Number	112-86-7
EC Number	204-011-3
Molecular Formula	$C_{22}H_{42}O_2$
Molecular weight	338.56768 g/mol
Boiling point	265°C at 15 mmHg
Melting point	33.8°C
Solubility in water	Insoluble: 2.66 × 10^{-4} mg dissolve in 1 L
Solubility in ethanol	Soluble: 175 g dissolve in 100 mL
Density	0.860 g/cm^3
Index of refraction	1.4534 at 45°C

CAS: Chemical Abstracts Service; EC: European Commission.

Erucic acid is present in seeds and oils mainly as a component of triacylglycerol molecules. These consist of a glycerol moiety in which each of the three hydroxyl groups is esterified with the carboxyl group of a fatty acid. The three hydroxyl groups of glycerol are designated as *sn*-1, *sn*-2 and *sn*-3, with *sn*-2 being the central one (Belitz et al., 2009). In the seed oils of some plant species containing erucic acid, this fatty acid is virtually excluded from the *sn*-2 position of the triacylglycerols, whereas in other species there is a balanced distribution of erucic acid in the three positions of the triacylglycerol molecule. For example, rapeseed oil contains a very low proportion of erucic acid at the *sn*-2 position, while *B. oleracea* and *Tropaeolum majus* seed oils contain similar levels of erucic acid at the three positions (Taylor et al., 1994). Such differences might have implications from a nutritional perspective, since it is well known that stereospecificity and chain length of fatty acids at the *sn*-1, *sn*-2 and *sn*-3 positions of triacylglycerols is a major determinant of the metabolic route that will follow fatty acids during digestion and absorption (Karupaiah and Sundram, 2007). However, such implications have not been studied in the particular case of erucic acid.

Erucic acid is also present in lipid classes other than triacylglycerol, e.g. diacylglycerols, free fatty acids, sterol esters, glycolipids and phospholipids. However, the proportion of erucic acid in these lipids in relation to other fatty acids is lower than in triacylglycerols, particularly in the case of phospholipids (Zadernowski and Sosulski, 1978). Studies in crambe have shown that the limited presence of erucic acid in the membrane lipids may be caused by a low phosphatidylcholine-diacylglycerol interconversion (Guan et al., 2014).

1.3.4. Analytical methods

Since erucic acid is a fatty acid, it is analysed using methods for fatty acids analysis. The Commission Directive 80/891/EEC[13] of 25 July 1980 laid down a Community method of analysis for determining the erucic acid content in oils and fats and in foodstuffs containing oils and fats. This Directive was repealed by Commission Regulation (EU) 2015/705[14] of 30 April 2015 as the method of analysis of erucic acid had become obsolete. Several associations and institutions have developed protocols that can be used for the analysis of the fatty acid composition in biological samples and food products. A list of relevant methods is given in Appendix B and the general principles are briefly described below.

[12] https://pubchem.ncbi.nlm.nih.gov/compound/erucic_acid#section=Top

[13] Commission Directive 80/891/EEC of 25 July 1980 relating to the Community method of analysis for determining the erucic acid content in oils and fats intended to be used as such for human consumption and foodstuffs containing added oils or fats. OJ L 254, 25.7.1980, p. 35–41.

[14] Commission Regulation (EU) 2015/705 of 30 April 2015 laying down methods of sampling and performance criteria for the methods of analysis for the official control of the levels of erucic acid in foodstuffs and repealing Commission Directive 80/891/EEC. OJ L 113, 1.5.2015, p. 29–37.

Extraction of lipids

Lipids in plant and animal tissues occur in different forms. Most oilseeds accumulate the large majority of the lipids in the form of fluid triacylglycerol droplets termed oil bodies, which are extracted easily with solvents such as hexane or diethyl ether. But on the other hand, membrane lipids occur in close association with other molecules such as polysaccharides and proteins. In these cases, lipid extraction requires not only dissolving the lipids, but mainly breaking the complex interactions with other membrane compounds. The most commonly used solvent combination for lipid extraction is chloroform–methanol (2:1 by volume) (Dijkstra et al., 2007). Extraction with supercritical fluids is a promising alternative to conventional methods based on organic solvents (Martinez and de Aguiar, 2014).

Gas chromatography-derived methods

The most common procedure for analysis of the fatty acid profile is derivatisation of fatty acids followed by separation and identification by gas chromatography (GC). Derivatisation consists in the transesterification of the carboxylic groups of the fatty acids, which reduces their polarity and makes them more amenable to analysis by GC. In the reaction of transesterification, a triacylglycerol reacts with an alcohol in the presence of a catalyst, usually a strong acid or base, producing a mixture of fatty acid alkyl esters and glycerol, and mono- and diacylglycerols are formed as intermediates (Knothe et al., 2007). There is a number of derivatisation procedures that differ in the alcohol used, type of catalyst used, and in parameters such as time of reaction, temperature, etc. (Christie, 1993). The most common procedures in most internationally approved methods are based on the use of an acidic catalyst and methanol as alcohol, which results in the formation of fatty acid methyl esters (FAMEs). For non-polar lipids, such as triacylglycerols, an additional solvent, such as toluene, should be added to facilitate their solubility (Dijkstra et al., 2007).

FAMEs are separated almost exclusively in capillary columns with polar polyester coatings. FAMEs are separated in the column according to their chain length, number of double bonds, position of the double bonds, and even the *cis/trans* configuration of double bonds. A polyester phase with low to medium polarity is generally adequate for the analysis of common fatty acids from plant and animal origin. In most cases, identification of erucic acid is simply conducted by comparing the retention time with that of an analytical standard. Flame ionisation detectors (FID) are commonly used for fatty acid analysis (Dijkstra et al., 2007). Exceptionally, it may be necessary to perform a confirmatory identification using mass spectrometry (MS) detection. There are two derivatisation procedures particularly well suited for MS detection of fatty acids, based on 3-hydroxymethylpyridinyl (picolinyl) esters and 4,4-dimethyloxazoline derivatives, respectively (Christie, 1998). MS also allows identifying positions of branching and in some cases the positions of unsaturation in the molecule (Dodds et al., 2005).

High-performance liquid chromatography-derived methods

Nearly all internationally recognised protocols for analysis of fatty acids are based on GC. However, methods for fatty acid analysis by reversed-phase high-performance liquid chromatography (HPLC) are also available. They are particularly useful to recover specific fractions for further characterisation. HPLC analysis also reduces the risk of isomerisation of unsaturated fatty acids. The analysis may be conducted with or without previous derivatisation of fatty acids, using a ultraviolet–visible photodiode array detector (Guarrasi et al., 2010).

Nuclear magnetic resonance spectroscopy

Both ^{1}H- and ^{13}C-nuclear magnetic resonance (NMR) spectroscopy are powerful techniques to elucidate structural properties of fatty acids (Dijkstra et al., 2007). Procedures for determining the fatty acid composition of oil samples by ^{1}H-NMR analysis have also been reported (Knothe and Kenar, 2004; Barison et al., 2010).

Near-infrared, Fourier transform near-infrared and attenuated total reflection-Fourier transform infrared spectroscopy

Both near-infrared (NIR) spectroscopy and Fourier transform (FT) NIR spectroscopy are secondary techniques for measuring the fatty acid profile that requires calibration using laboratory data provided by a primary technique, usually GC analysis of FAMEs (Velasco and Becker, 1998; Kolackova et al., 2014). Both techniques are less accurate than GC analysis, as they accumulate the prediction error to

their own analytical error, but have the main advantage of allowing very fast analyses without any kind of sample preparation. NIR and FT-NIR are not adequate when an accurate analysis of the fatty acid profile is required, but they are extremely useful for screening, real-time monitoring of processes or *in-situ* measurements (Pérez-Marín et al., 2009). Attenuated total reflection-Fourier transform infrared (ATR-FTIR) spectroscopy is also a technique used for analysis of erucic acid content in vegetable oils (Sherazi et al., 2013) that, additionally, has the great advantage of being suitable for non-invasive measurement of fatty acid composition in biological tissues (Yoshida, 2008).

Analytical quality assurance: performance criteria, reference materials and proficiency testing

Performance criteria for the analytical methods used for the official control of levels of erucic acid in foodstuffs are laid down in Commission Regulation (EC) No 2015/705. Whereas no specific methods for the determination of erucic acid content in foodstuffs are prescribed at the EU level, laboratories may select any validated method of analysis for the respective matrix, provided that the selected method meets the specific performance criteria set out in the Regulation. It is recommended that fully validated methods, i.e. validated by collaborative trial, are used where appropriate and available. Proficiency testing schemes including erucic acid are conducted regularly by organisations such as The International Bureau for Analytical Studies (BIPEA),[15] Fapas,[16] or The American Oil Chemists' Society (AOCS)[17] among others. Reference materials are available from these organisations and others such as the National Institute of Standards and Technology (NIST).[18] Other suitable validated methods, e.g. in-house validated methods may also be used provided that they fulfil the performance criteria set out in the Regulation. The Regulation follows a criteria-centred approach based on a given number of method characteristics taken individually. The limit of detection (LOD) is required to be less than 1 g/kg fat and the limit of quantification (LOQ) to be less than 5 g/kg fat (Commission Regulation (EC) No 2015/705). Typical LOD and LOQ values reported in the literature using GC and FID detection, which is the most common detection method in routine analyses, are around 0.03 and 0.1 g/kg fat, respectively (Dixit and Das, 2012). Much lower values are reported for GC methods based on more sensitive MS detectors (Devle et al., 2009). According to the Commission Regulation, the recovery should be between 95% and 105%.

1.3.5. Legislation

In order to protect public health, Article 2 of Council Regulation (EEC) No 315/93 stipulates that, where necessary, maximum tolerances for specific contaminants shall be established.[19] Currently, maximum levels (MLs) for erucic acid are laid down in the Annex, Section 8, of Commission Regulation (EC) No 1881/2006. An ML of 50 g erucic acid/kg fat[20] applies to vegetable oils and fats, and foods containing added vegetable oils and fats with the exception of infant formulae and follow-on formulae. For infant formulae and follow-on formulae an ML of 10 g erucic acid/kg fat applies.[20]

2. Data and methodologies

2.1. Data

2.1.1. Occurrence data

2.1.1.1. Data collection and validation

At the moment of receiving the request for the scientific opinion from the European Commission (EC), no data on erucic acid (22:1 n-9) were available in the EFSA Chemical Occurrence database. The EFSA Evidence Management Unit (DATA Unit) initiated an ad-hoc collection of data to compile occurrence data on erucic acid levels in food and feed.[21] The European national food authorities and similar bodies, research institutions, academia, and food business operators were invited to submit

[15] http://www.bipea.org/
[16] http://fera.co.uk/fapas-proficiency-testing/
[17] http://www.aocs.org/LabServices/content.cfm?ItemNumber=841&navItemNumber=640
[18] http://www.nist.gov/
[19] In this scientific opinion, where reference is made to European legislation (regulations, directives, decisions), the reference should be understood as relating to the most current amendment, unless otherwise stated.
[20] The maximum level refers to the level of erucic acid, calculated on the total level of fatty acids in the fat component in food.
[21] http://www.efsa.europa.eu/en/dataclosed/call/150408

data. The data for the present assessment were provided by national authorities from Austria, Cyprus, the Czech Republic, Denmark, France, Germany, Hungary, Poland, Slovenia, Slovakia and the UK, and by the European Vegetable Oil and Proteinmeal Industry (FEDIOL), by the Specialised Nutrition Europe (SNE) and by the European Feed Manufacturers' Federation (FEFAC).

The data submission to EFSA followed the requirements of the EFSA Guidance on Standard Sample Description for Food and Feed (EFSA, 2010a); occurrence data were managed following the EFSA standard operational procedures (SOPs) on 'Data collection and validation' and on 'Data analysis of food consumption and occurrence data'.

By the end of December 2015, a total of 16,671 samples of food, drinking water and feed with analytical data on erucic acid were available in the EFSA database. Approximately 2% of the samples were reported as feed and the rest as food and drinking water samples. Data received after that date were not included in the initial dataset used to estimate dietary exposure.

2.1.1.2. Data analysis

Following the EFSA SOP on 'Data analysis of food consumption and occurrence data' to guarantee an appropriate quality of the data used in the exposure assessment the initial dataset was carefully evaluated applying several data cleaning and validation steps. Special attention was paid to different parameters such as 'Sampling strategy', 'Sampling year', 'Sampling country', 'Analytical methods', 'Reporting unit' and the codification of the different samples under FoodEx classification (for food) and Commission Regulation No 68/2013[22] (for feed), among others. The outcome of the data analysis is shown in Section 3.1.2.

Analytical results were reported either in whole weight basis or fat basis. Before estimating dietary exposure, all results were converted into mg/kg whole weight. For those samples expressed on fat weight basis, the fat content was used to convert the analytical result into whole weight; when fat content was missing, whenever possible imputation of the fat content from reported values was done (see Section 3.1.2).

The left-censored data (analytical data below the LOD/LOQ) were treated by the substitution method as recommended in the 'Principles and Methods for the Risk Assessment of Chemicals in Food' (WHO/IPCS, 2009). The same method is indicated in the EFSA scientific report 'Management of left-censored data in dietary exposure assessment of chemical substances' (EFSA, 2010b) as an option in the treatment of left-censored data. The guidance suggests that the lower bound (LB) and upper bound (UB) approach should be used for chemicals likely to be present in the food (e.g. naturally occurring contaminants, nutrients and mycotoxins). At the LB, results below the LOQ or LOD were replaced by zero; at the UB, the results below the LOD were replaced by the LOD and those below the LOQ were replaced by the value reported as LOQ. Additionally, a middle bound (MB) approach was also used by assigning a value of LOD/2 or LOQ/2 to the left-censored data. The use of different cut-off values on the reported LOQs was also evaluated in order to reduce the uncertainty associated to the exposure estimations.

2.1.2. Consumption data

2.1.2.1. Food consumption data

The EFSA Comprehensive European Food Consumption Database (Comprehensive Database) provides a compilation of existing national information on food consumption at individual level. It was first built in 2010 (EFSA, 2011a; Huybrechts et al., 2011; Merten et al., 2011). Details on how the Comprehensive Database is used are published in the Guidance of EFSA (EFSA, 2011a). The latest version of the Comprehensive Database[23] was used and contains results from a total of 51 different dietary surveys carried out in 23 different Member States covering 94,532 individuals.

Within the dietary studies, subjects are classified in different age classes as follows:

Infants: < 12 months old
Toddlers: ≥ 12 months to < 36 months old
Other children: ≥ 36 months to < 10 years old
Adolescents: ≥ 10 years to < 18 years old
Adults: ≥ 18 years to < 65 years old

[22] Commission Regulation (EU) No 68/2013 of 16 January 2013 on the Catalogue of feed materials. OJ L 29, 30.1.2013, p. 1–64.
[23] http://www.efsa.europa.eu/en/datexfoodcdb/datexfooddb

Elderly: ≥ 65 years to < 75 years old
Very elderly: ≥ 75 years old

Two additional surveys provided information on specific population groups: 'Pregnant women' (≥ 15 years to ≤ 45 years old; Latvia) and 'Lactating women' (≥ 28 years to ≤ 39 years old; Greece).

For chronic exposure assessment, food consumption data were available from 44 different dietary surveys carried out in 19 different European countries. When for one particular country and age class two different dietary surveys were available, only the most recent one was used. This result in a total of 35 dietary surveys selected to estimate chronic dietary exposure. In Appendix C, Table C.1, these dietary surveys and the number of subjects available for the chronic exposure assessment are described.

Overall, the food consumption data gathered by EFSA in the Comprehensive Database are the most complete and detailed data currently available in the EU. Consumption data were collected using single or repeated 24- or 48-h dietary recalls or dietary records covering from 3 to 7 days per subject. Owing to the differences in the methods used for data collection, direct country-to-country comparisons can be misleading.

2.1.2.2. Feed consumption data

There is considerable variation in both the feeds used and the feeding systems adopted for farmed livestock and companion animals throughout the EU. This variation is largely due to the availability of feeds and market demands for specific animal products, the quality of the feeds available and nutritional needs of the animals concerned.

Estimating the exposure to erucic acid requires estimates of feed consumed and levels of erucic acid in feed. In the absence of an EU database on feed consumption by livestock and companion animals, estimates of feed intake have been derived from published guidelines on nutrition and feeding (AFRC, 1993; Carabano and Piquer, 1998; NRC, 2007a,b; Leeson and Summers, 2008; McDonald et al., 2011; EFSA FEEDAP Panel, 2012; OECD, 2013), data on EU manufacture of compound feeds[24] and expert knowledge of production systems in Europe. In addition, maximum recommended levels of rapeseed meal and rapeseed oil have been taken from feed industry publications (Ewing, 1997; Canola Council of Canada, 2015). Details are given in Appendix D. It should be stressed that these do not represent 'average' feed intakes or diets, nor are the feeding systems 'typical' for all of Europe. Rather, the approach adopted here provides likely upper limits of exposure to erucic acid.

2.1.2.3. Food classification

Consumption data were classified according to the FoodEx classification system (EFSA, 2011b). FoodEx is a food classification system developed by EFSA in 2009 with the objective of simplifying the linkage between occurrence and food consumption data when assessing the exposure to hazardous substances. It contains 20 main food groups (first level), which are further divided into subgroups having 140 items at the second level, 1,261 items at the third level and reaching about 1,800 endpoints (food names or generic food names) at the fourth level.

In 2011, a new version of FoodEx, named FoodEx2 has been developed and is described in the scientific document 'Report on the development of a Food Classification and Description System for exposure assessment and guidance on its implementation and use' (EFSA, 2011c). The last release of FoodEx2 complements the previous hierarchical classification system of basic codes with more detailed food levels and gives the possibility of reporting additional information through the use of facets and facet descriptors (EFSA, 2015).

2.1.3. Toxicokinetic and toxicological data

With the exception of one document provided to EFSA, all data were obtained from the scientific literature as described in Section 2.2.2.

[24] www.fefac.eu

2.2. Methodologies

2.2.1. Dietary exposure assessment

2.2.1.1. Dietary exposure assessment in humans

The CONTAM Panel considered that only chronic dietary exposure to erucic acid had to be assessed (see Section 3.3.6). As suggested by the EFSA Working Group on Food Consumption and Exposure (EFSA, 2011a), dietary surveys with only 1 day/subject were not considered as they are not adequate to assess repeated exposure. Similarly, subjects who participated only 1 day in the dietary studies, when the protocol prescribed more reporting days per individual, were also excluded for the chronic exposure assessment. Thus, for chronic exposure assessment, food consumption data were used from 35 different and most recent dietary surveys carried out in 19 different European countries present in the latest version of the Comprehensive Database (Appendix C, Table C.1).

For calculating chronic dietary exposure to erucic acid, food consumption and body weight data at the individual level were accessed in the Comprehensive Database. Occurrence data and consumption data were linked at the lowest (most detailed) FoodEx level possible. In addition, the different food commodities were grouped within each food category to better explain their contribution to the total dietary exposure to erucic acid. Exposure estimates were calculated for each dietary survey and age class. Not all countries provided consumption information for all age groups, and in some cases, the same country provided more than one consumption survey. The mean and the high (95th percentile) chronic dietary exposures were calculated by combining erucic acid mean occurrence values for food samples collected in different countries (pooled European occurrence data) with the average daily consumption for each food at individual level in each dietary survey.

All analyses were run using the SAS Statistical Software (SAS enterprise guide 5.1).

2.2.1.2. Dietary exposure assessment in animals

Animal exposure to erucic acid by animals was determined by its concentration in the feed and the quantity of feeds consumed. In the absence of data on concentrations of erucic acid in compound feeds, the CONTAM Panel has used industry data on levels of inclusion of rapeseed meal and rapeseed oil for livestock, fish and companion animals. Details of feed intakes and levels of erucic acid used in estimating animal exposure to erucic acid are given in Appendix D.

2.2.2. Literature search and appraisal of studies

2.2.2.1. Strategy for literature search

A comprehensive search for literature was conducted for peer-reviewed original research pertaining to adverse health effects on animals (experimental, livestock, horses and pets) and humans. The search strategy was designed to identify scientific literature dealing with toxicity, mode of action, toxicokinetics and human data on erucic acid. An overview of the search terms is given in Appendix E, Section E.1.

It should be noted that a narrative approach was used for those sections dealing with methods of analysis, chemistry, occurrence and exposure since the identified papers are only used to give background information to the reader.

The literature search was not restricted to publications in English. A first literature search was performed in April 2015 (March 2016 for papers on the occurrence in human milk) and has been updated in May 2016 to identify papers dealing with toxicity, mode of action, toxicokinetics and human data. Web of Science[25] and PubMed[26] were identified as databases appropriate for retrieving literature for the present evaluation. The references obtained from the literature search were imported and saved using a software package (EndNote[27]). Additionally, reviews, relevant scientific evaluations by national or international bodies were considered for the current risk assessment, i.e. previous

[25] Web of Science (WoS), formally ISI Web of Knowledge, Thomson Reuters. Available at: http://thomsonreuters.com/thomson-reuters-web-of-science/

[26] PubMed, Entrez Global Query Cross-Database Search System, National Center for Biotechnology Information (NCBI), National Library of Medicine (NLM), Department of the National Institutes of Health (NIH), United States Department of Health and Human Services. Available at: http://www.ncbi.nlm.nih.gov/pubmed/

[27] EndNote X5, Thomson Reuters. Available at: http://endnote.com/

evaluations of FSANZ (2003) and SCF (1995, 2003). In addition, when relevant papers were identified during the risk assessment process (e.g. from other studies or reviews) they were also considered.

The references obtained were screened using title and abstract to identify the relevant literature and exclusion criteria are shown in Appendix E, Section E.2.

2.2.2.2. Appraisal of studies

Information retrieved has been reviewed by the CONTAM working group (WG) on erucic acid in food and feed, and has been used for the present assessment based on expert judgement. Any limitations in the information used are documented in this scientific opinion.

Selection of the scientific papers for inclusion or exclusion was based on consideration of the extent to which the study was relevant to the assessment and general study quality considerations (e.g. sufficient details on the methodology, performance and outcome of the study, on dosing and route of administration and on statistical description of the results (EFSA, 2009), irrespective of whether they yielded positive, negative or null results).

Toxicological studies in experimental animals which did not provide information on the erucic acid concentration in the administered material were excluded.

All information retrieved as described in the previous paragraph has been reviewed and used for the present assessment using expert judgement.

It should be noted that no comprehensive literature search, appraisal of the studies and reporting was carried out for scientific literature dealing with methods of analysis, chemistry, occurrence and exposure since the identified papers are only used to give background information for the reader.

2.2.3. Methodology applied for risk assessment

The CONTAM Panel applied the general principles of the risk assessment process for chemicals in food as described by WHO/IPCS (2009), which include hazard identification and characterisation, exposure assessment and risk characterisation. Additionally to the principles described by WHO/IPCS (2009), EFSA guidance pertaining to risk assessment has been applied for the present assessment (see Appendix E, Section E.3).

The evaluation of the inherent uncertainties in the assessment of exposure to erucic acid has been performed following the guidance of the Opinion of the Scientific Committee related to Uncertainties in Dietary Exposure Assessment (EFSA, 2006). In addition, the report on 'Characterizing and Communicating Uncertainty in Exposure Assessment' has been considered (WHO/IPCS, 2008). According to the guidance provided by the EFSA opinion (2006), the following sources of uncertainties have been considered: assessment objectives, exposure scenario, exposure model, model input (parameters) and other uncertainties.

3. Assessment

3.1. Occurrence data

3.1.1. Previously reported occurrence data

3.1.1.1. Food

Since erucic acid is a fatty acid of which the occurrence is reported in food nutrients databases, the CONTAM Panel consulted four comprehensive food nutrients databases (Max Rubner Institut, 2010; NFI, 2015; Public Health England, 2015; US Department of Agriculture, 2015) to collect previously reported occurrence data. In total, 12,934, 417, 533 and 5,487 entries, respectively, have been checked for occurrence of erucic acid. Information from scientific literature has been retrieved only in particular cases to fill in gaps found in the databases or in relation to food types of particular interest for this opinion. Appendix F provides for the different food groups examples of the occurrence of erucic acid in foods. When the amount of erucic acid per unit of weight of the sample was available or could be computed, it has been converted to mg/kg. Sources providing erucic acid as percentage of total fatty acids but not the lipid content of the sample have not been considered. Foods were grouped following the FoodEx classification (EFSA, 2011b). Food groups in which no erucic acid is reported are included as footnotes in the tables in Appendix F.

Erucic acid mainly occurs in oil seeds from the Brassicaceae family (see Section 1.3.1). Since these oil seeds and the derived oils are used in a wide variety of foods, erucic acid occurs in many different

food groups. Besides the occurrence in oil seeds, erucic acid also occurs naturally in fish and seafood. These food groups mainly contain cetoleic acid (22:1 n-11), which is usually accompanied by minor proportions of erucic acid (Ackman, 2008). It is important to note that both fatty acids are not easily separated and identified under non-optimal analytical conditions (Ackman, 2008), which leads to relatively common misidentifications in food nutrients databases and scientific literature, as detailed below.

It is also of importance that the database of the Max Rubner Institut provides a single value for 22:1 fatty acids, with no distinction between erucic acid (22:1 n-9) and cetoleic acid (22:1 n-11) or between *cis* and *trans* isomers. Similarly, the US Department of Agriculture database does not differentiate between erucic acid and cetoleic acid, and it reports *cis* 22:1 as the sum of both 22:1 n-9 and 22:1 n-11. Therefore, information from these databases has not been included in Appendix F, Table F.7 (Fish and other seafood). For the other tables this is not a major problem since erucic acid is the only 22:1 isomer present in vegetable food and 22:1 n-11 isomers are in general not present in non-marine animal food (Chow, 2008). The exceptions are food samples containing partially hydrogenated oils that can contain positional isomers of 22:1. In these cases, a slight overestimation of erucic acid can be expected in data from the database of the Max Rubner Institut and the US Department of Agriculture. The database of Public Health England reports separately *cis* + *trans* 22:1 n-9 and *cis* + *trans* 22:1 n-11. The tables in Appendix F only include data of *cis* + *trans* 22:1 n-9. Slight overestimation of the erucic acid concentration can be also expected in samples containing brassidic acid (*trans* 22:1 n-9). The database of the National Food Institute of Denmark contains data on 22:1 n-9. Nonetheless, the CONTAM Panel noted some putative fatty acid misidentifications in fish samples of the databases, which contained high levels of erucic acid (22:1 n-9) but no cetoleic acid (22:1 n-11). Those samples have not been taken into account in this Opinion because they did not follow the expected ratios of both isomers (Ackman, 2008), which might indicate wrongly identified fatty acids. For example, four samples of herring in the database of the National Food Institute of Denmark averaged 1,392 mg/kg for erucic acid and 27,035 mg/kg of cetoleic acid, while one sample had erucic acid content of 19,600 mg/kg and no cetoleic acid. Similarly, one sample of mackerel in the database of Public Health England contained both erucic acid (2,100 mg/kg) and cetoleic acid (34,300 mg/kg), but another sample contained much higher erucic acid content (27,900 mg/kg) and no cetoleic acid.

Grains and grain-based products (Appendix F, Table F.1) contain in general low concentrations of erucic acid, with the exception of several samples of biscuits, cakes and pastries with concentrations up to 24,660 mg/kg. Other foods with high levels of erucic acid within this food category included bread (up to 1,500 mg/kg), crackers (up to 1,170 mg/kg), doughnuts (up to 3,190 mg/kg), muffins (up to 1,040 mg/kg) and pancakes (up to 1,960 mg/kg). Relatively high concentrations of erucic acid (up to 1,066 mg/kg) have been also reported for quinoa seeds. Although quinoa is not a cereal, as it belongs to the Amaranthaceae family, the seeds have similar uses as cereals and they are accordingly considered as pseudo cereals. Quinoa is one of the few plant species out of the Brassicaceae family that can accumulate erucic acid in the seed, with a level typically below 2% of the total fatty acids (Wood et al., 1993).

In vegetables and vegetable products (Appendix F, Table F.2), the highest erucic acid concentration is found in sprouts from *Brassica* seeds rich in erucic acid. *Brassica* seedlings maintain a high oil concentration for several days after germination (i.e. around 64% of the initial value in dry seeds after 8 days) and practically unchanged proportion of erucic acid (Dawood et al., 2013). This makes sprouts from high erucic acid seeds rich sources of erucic acid. Other vegetable samples have levels of erucic acid less than 1,000 mg/kg.

Relatively low levels of erucic acid are generally found in starchy roots and tubers (Appendix F, Table F.3). The highest concentration (up to 4,290 mg/kg) corresponded to French fries.

In the food category of legumes, nuts and oilseeds (Appendix F, Table F.4), the highest erucic acid concentration corresponds to seeds of the Brassicaceae family, mainly *Brassica* spp. (rapeseed, turnip rape, mustards, crambe), with erucic acid concentration up to 200,000 mg/kg. High erucic acid concentrations, up to 42,744 mg/kg, have also been reported for borage seeds. The highest erucic acid concentration in legume seeds is found in lupine and cowpea seeds, in which erucic acid typically represents up to 3% of the total fatty acids (Bhardwaj et al., 2004; Antova et al., 2014). Within the different lupine species, the highest erucic acid concentrations are usually found in white lupine (*Lupinus albus* L.), whose seeds are a popular snack in the Mediterranean region (Cowling, 2001). In nuts, the highest erucic acid concentration (2,370 mg/kg) was found in a sample of macadamia nuts. Considering that macadamia does not accumulate naturally erucic acid in the nuts (Maguire et al.,

2004), the erucic acid present in this sample is expected to originate from the oil used for roasting the nuts.

In general, erucic acid is not found in fruit and fruit products (Appendix F, Table F.5). In meat samples (Appendix F, Table F.6), erucic acid is generally not detected or present at low levels, with some exceptions such as soy sausages showing an erucic acid content of up to 4,020 mg/kg. The highest erucic acid concentrations in fish and other seafood (Appendix F, Table F.7) have been reported in samples of fish pâté and halibut (4,300 mg/kg), salmon (3,990 mg/kg), herring (2,480 mg/kg), sprat (2,390 mg/kg) and mackerel (2,190 mg/kg).

Low levels of erucic acid are generally present in the food categories milk and dairy products (Appendix F, Table F.8) and sugar and confectionary (Appendix F, Table F.9), with the highest concentration in a sample of cheese (400 mg/kg) in the former and a sample of chocolate bar (910 mg/kg) in the latter.

Among animal and vegetable fats and oils (Appendix F, Table F.10), oils from high erucic acid cultivars of mustard and rapeseed have the highest concentrations of erucic acid (above 500,000 mg/kg). Conversely, oils from low erucic acid rapeseed cultivars, also known as canola, had much lower concentrations of erucic acid, up to 2,410 mg/kg. Some oil samples with a high erucic acid concentration from seeds or fruits such as corn, cottonseed, flaxseed, palm, peanut, safflower, sesame and sunflower that do not accumulate erucic acid (Gunstone and Harwood, 2007), are most likely the result of oil adulterations or analytical misidentifications. In the UK, the Food Standards Agency (FSA) reported concentrations up to 270,000 mg/kg in the oil from pickle samples. These samples have been taken from pickles imported from Bangladesh, China, India and Pakistan (FSA, 2004), which are countries in which the use of high erucic acid oil for food is still common. High erucic acid concentrations are also reported for samples of shortenings (up to 58,740 mg/kg), margarine (up to 45,980 mg/kg) and butter (up to 9,560 mg/kg).

In general, no erucic acid is found in fruit and vegetable juices (Appendix F, Table F.11). In non-alcoholic beverages (Appendix F, Table F.12), two samples of cocoa drinks contained erucic acid up to 3,450 mg/kg. The group of herbs, spices and condiments (Appendix F, Table F.13) contains food products in which erucic acid is found at high concentrations, mainly mustards (up to 20,790 mg/kg) and to a lesser extent mayonnaise (up to 3,100 mg/kg), sauces, salad dressings and coleslaw (up to about 1,000 mg/kg). The reason for the high erucic acid content in mustard samples is that mustard is mainly produced from high erucic acid-containing *Brassica juncea* and *Sinapis alba* seeds, where between 25% and 35% of the total fatty acids is erucic acid (Siemens, 2014). Mustard is therefore one of the food products with the highest erucic acid content that can be found in European markets.

Low erucic acid levels were identified in food for infants and small children (Appendix F, Table F.14) and products for special nutritional use (Appendix F, Table F.15). Composite dishes (Appendix F, Table F.16) have in general a low erucic acid content, but there are some notable exceptions, e.g. 11,000 mg/kg in a sample of grilled salmon (farmed) with 15.6% fat content, in which high levels of cetoleic acid were also present (96,000 mg/kg), or 20,000 mg/kg in a sample of roasted chicken skin, in which high erucic acid oil was probably used for roasting. Apart from these samples, the highest concentrations (about 5,000 mg/kg) corresponded to meat- and vegetable-based dishes. In the food category of snacks, desserts and other foods (Appendix F, Table F.17), the highest levels of erucic acid (up to 3,190 mg/kg) are found in milk-based desserts. No erucic acid is found in eggs and egg products, alcoholic beverages, and drinking water.

In addition, notifications on the occurrence of erucic acid in different food commodities are reported in the Rapid Alert System for Food and Feed (RASFF). RASFF is a key tool to ensure the cross-border follow of information to swiftly react when risks to public health are detected in the food chain. Around 50 notifications have been made since 2003 notifying high levels of erucic acid in diverse food commodities. The occurrence data largely refer to levels of erucic acid in samples of pickles (pickles and its oil) and mustard oil coming from Asian countries such as Bangladesh, India and Pakistan. As an example, erucic acid levels up to 49.3% have been reported in mustard oil from Bangladesh or up to 56.2% in samples reported as mango hot pickle, also from Bangladesh.

Human milk

Milk is a complex fluid containing around 3-5% lipids that mainly occur as globules emulsified in the aqueous phase. About 98% of the human milk lipids are triacylglycerols, the remaining part being mainly phospholipids and cholesterol (Jensen, 1999). The maternal diet is the main factor influencing the fatty acid composition of the milk lipids, although other factors such as time post-partum, gestational age, parity (number of pregnancies and number of pregnancies carried to a viable

gestational age) or diseases also can influence the fatty acid profile (Jensen, 1999). Table 3 provides examples of levels of erucic acid in human milk from European women. In general, the percentage erucic acid of the total fatty acids in human milk ranged from 0.06% to 0.22% (Table 3). However in one study with Greek women, lower percentages (0.001–0.004%) were reported.[28] The highest percentages (≥ 0.18%) were observed at the beginning of lactation (Precht and Molkentin, 1999; Marangoni et al., 2000; López-López et al., 2002; Mihalyi et al., 2015).

Table 3: Levels of erucic acid in human milk reported for different European countries

Country	N	Percentage erucic acid of total fatty acids	Concentration of erucic acid in human milk (mg/L)[(d)]	References
United Kingdom	44	0.10 ± 0.00[(a)]	–[(e)]	Yuhas et al. (2006)
The Netherlands	21–44	0.10 ± 0.03–0.11 ± 0.04[(b)]	35–36.3	Beijers and Schaafsma (1996)
Poland	20–136	0.13 ± 0.07–0.14 ± 0.09[(b)]	38.9–39.0	Szlagatys-Sidorkiewicz et al. (2013)
Germany	40	0.18 ± 0.06[(b)]	52.2	Precht and Molkentin (1999)
Hungary	46–87	0.07–0.18[(c)]	–[(e)]	Mihalyi et al. (2015)
France	10	0.12 ± 0.03[(a)]	–[(e)]	Chardigny et al. (1995)
Italy	10–95	0.06 ± 0.03–0.22 ± 0.08[(b)]	–[(e)]	Marangoni et al. (2000)
Portugal	31	0.06 ± 0.01–0.11 ± 0.02[(b)]	–[(e)]	Ribeiro et al. (2008)
Spain	120	0.06 ± 0.03–0.18 ± 0.11[(b)]	–[(e)]	López-López et al. (2002)
Spain	8–12	0.11 ± 0.02–0.16 ± 0.03[(b)]	–[(e)]	Rueda et al. (1998)
Greece	24–64	0.0013 ± 0.061–0.004 ± 0.02[(b)]	0.34–1.27	Antonakou et al. (2013)

N: number of samples.
(a): Mean ± standard error.
(b): Mean ± standard deviation.
(c): Standard error and standard deviation not reported.
(d): Concentration calculated based on fat content and percentage of erucic acid.
(e): Concentration of erucic acid in human milk was not calculated due to lack of information.

3.1.1.2. Feed

Erucic acid is a component of the oil seed and after oil extraction, the remaining meal is widely used as a protein source in animal feeds. Even under industrial oil extraction conditions, a small proportion of the oil remains in the meal. Rapeseed meal typically contains 3.5% of oil on a 12% moisture basis, although this depends on the extraction conditions (Newkirk, 2009). Therefore, the erucic acid content in the meal will depend on the amount of remaining oil in the meal and the proportion of erucic acid in the oil. Rapeseed meal used for livestock feed in the EU is produced using the double-zero varieties, in which oil contains < 5% erucic acid (Commission Regulation (EC) No 1881/2006) and typically oils with an erucic acid content at or below 0.5% are used (see Appendix A, Table A.1). Since the oil content of rapeseed meal is typically 3.5%,[29] the erucic acid content of the meal is likely to be < 175 mg/kg. For example, a meal containing 3.5% oil from a modern rapeseed cultivar with low erucic acid content (e.g. 0.1%) will contain only 35 mg erucic acid/kg meal. Some whole rape seed is also used as feed for livestock.

3.1.2. Current occurrence data

3.1.2.1. Food

An initial number of 16,401 food samples with analytical data on erucic acid were available. As described in Section 2.1.1, the occurrence data were carefully analysed before being used to estimate dietary exposure. A total of 3,957 food samples were excluded from the final dataset as described in Appendix C, Table C.2.

[28] Data confirmed by the authors.
[29] www.feedipedia.org

Briefly, 1,729 samples of diverse types of oil (coconut oil, almond oil, corn oil, grape seed oil, linseed oil, olive oil, palm oil, soybean oil, sunflower oil, safflower oil, walnut oil and wheat germ oil) were excluded from the final dataset as these oils should not contain naturally erucic acid (Gunstone and Harwood, 2007). Most of the samples (87%) belonged to three types of oil, olive oil (n = 515), sunflower oil (n = 478) and pumpkinseed oil (n = 328) of which 95% was left-censored. Mean LB erucic acid levels in these samples ranged from 5 (in pumpkinseed oil) to 146 mg/kg (in sunflower oil) and UB levels from 44 (in pumpkinseed oil) to 880 mg/kg (in olive oil). In the remaining types of oil (13% of the total number of samples) the highest number of quantified samples was reported for safflower oil (26 out of 68 samples). Mean erucic acid levels were between 21 mg/kg (LB) in palm oil and 1,440 mg/kg (UB) in wheat germ oil. A special case refers to 'Peanut oil' for which 70% of the samples (n = 96) reported levels of erucic acid. Although peanut oil does not contain erucic acid naturally (Carrín and Carelli, 2010), the high amount of quantified samples seems to indicate an extensive presence of adulterated peanut oil in the market. Moreover, the main producing countries of rapeseed oil are also high producers of peanut oil. Based on this fact, the CONTAM Panel decided to keep the samples of peanut oil in the final dataset. Additionally, 415 samples of vegetable oil reported without further information on the seed/fruit used to extract the oil were also excluded.

The results of 1,783 samples were reported on fat weight basis without information on fat content. Almost 800 of these samples were kept in the final dataset, as the fat content was imputed by using the fat content of similar samples or the EFSA composition database (mainly oil samples, food for infants and few samples of fish). For around 1,000 samples, the imputation was not possible as they refer to foods subjected to a high variation in their fat content and, therefore, the assignment of a fat content would largely increase the uncertainty.

The presence of a high percentage of left-censored data together with high left-censoring limits can provoke substantial differences between LB and UB scenarios increasing the uncertainty associated to the dietary exposure estimations. Based on the EFSA internal guidance on the application of LOD/LOQ cut-offs, special attention was paid to food groups for which the difference between LB and UB was higher than 40% and that were relevant for the exposure. Two main food groups were identified, 'Grain and grain-based products, in particular 'Pastries and cakes' and 'Biscuits (cookies)' and 'Ready-to-eat meal for infants and young children'. By analysing both distributions, that of the reported LOQs and that of the quantified results, several values were selected and subsequently applied as cut-offs to the LOQs reported for these food groups. A total of 178 samples were excluded following this criterion. Appendix C, Table C.3 shows the effect of these cut-offs on the occurrence values for the main selected food groups.

A total of 63 samples reported as 'Composite food' were also excluded from the final dataset. This food group, that represents ready-to-eat foods collected in shops, restaurants and processing plants, contained a very heterogeneous mix of samples with a very limited number of samples reported for each subgroup (see Appendix C, Table C.4). For some of these samples relatively high values of erucic acid were reported, indicating the use of rapeseed oil and/or specific herbs/spices/condiments in their preparation. The consumption data on composite foods contained in the Comprehensive Database does not allow knowing whether composite foods were prepared at home or bought in retails or consumed outside. This could have an influence on how the different composite foods were prepared (type of oil, herbs, spices, etc. used) and, therefore, in their levels of erucic acid. Then, the level of disaggregation of the reported foods is different depending on the dietary survey. It could be that countries which report a higher number of eating occasions for composite foods mainly refer to home-made foods while in dietary surveys with a higher level of disaggregation, the consumption refers to ready-to-eat foods. Therefore, considering the uncertainty regarding the consumption of composite food and the limited number of available occurrence data, the CONTAM Panel decided to exclude these samples from the final dataset used in the general exposure scenario. However, a particular exposure scenario was carried out combining some of the reported composite samples with consumption values derived from the Comprehensive Database in order to address the potential contribution of this type of food commodities on the exposure to erucic acid (see Section 3.2.1.2). Finally, two samples of 'Custard' were also excluded from the final dataset as the standard recipe for this dessert (milk, eggs, sugar) does not include ingredients that might contain erucic acid. The average value reported for these samples of 'Custard' was 1,010 mg/kg (LB=UB); a specific exposure scenario was also elaborated to estimate the potential exposure to erucic acid from the consumption of these custard samples (see Section 3.2.1.2).

After the quality assessment of the analytical data on erucic acid, a total of 12,444 food samples were available to estimate dietary exposure. Before the occurrence data were used to estimate dietary

exposure, the data were grouped at different FoodEx levels according to their erucic acid levels and the number of samples reported (Appendix C, Table C.5).

The highest number of available samples corresponded to the food group 'Animal and vegetable fats and oils' (~ 60%) and in particular to 'Rapeseed oil' (5,832 samples). Other food groups that were well represented were 'Starchy roots and tubers' (n = 1,223), 'Grains and grain-based products' (n = 982) and 'Food for infants and small children' (n = 810).

The highest individual value for erucic acid was reported for one sample of rapeseed oil (550,000 mg/kg), in line with maximum values reported in different databases as shown in Appendix F, Table F.10. Mean values were 1,285/5,215 mg/kg (LB/UB) and the large difference between LB and UB is probably due to the fact that among the 5,832 samples of rapeseed oil, more than 80% were left-censored data. The average value reported for the quantified samples (n = 1,117) was 6,712 mg/kg. For the left-censored data, an LOQ of 5,000 mg/kg was reported in 98% of the cases which is in line with the fact that current rapeseed varieties are characterised by very low erucic acid content, usually below 0.5% (Temple-Heald, 2004). It is worth mentioning that the average level of erucic acid among the quantified samples of peanut oil (n = 68) was 2,700 mg/kg, this may indicate an adulteration with HEAR oil.[30]

Only few samples (n = 50) of fish and fish products were available. Overall, the highest value of erucic acid was reported for one sample of 'Herrings' (7,200 mg/kg). The three main groups of fish samples were 'Tuna' (n = 18), 'Sardine and pilchard' (n = 14) and 'Salmon and trout' (n = 9). 'Sardine and pilchard' and 'Salmon and trout' samples contain similar average levels of erucic acid (LB = UB = ~ 1,000 mg/kg) while the levels reported for 'Tuna' were much lower (7/70 mg/kg (LB/UB)). The highest values of erucic acid among the samples of 'Sardine and pilchard' were reported for samples of sardines canned in oil; it cannot be excluded that part of the erucic acid comes from the oil even though the samples were reported as canned in 'olive oil'.

As mentioned in Section 3.1.1.1 no erucic acid is, in general, quantified in meat. Available samples for this scientific opinion mainly refer to meat imitates (soy protein), in particular to soy sausages that may have used rapeseed oil and/or different spices in their preparation, which may explain the erucic acid values detected (LB = UB = 900 mg/kg; Appendix C, Table C.5).

Relatively high values were reported for samples of mixed spices (mean LB = UB = 62,000 mg/kg) and for some mustard samples (mean LB = UB = 20,000 mg/kg) within the food group 'Herbs, spices and condiments'. Erucic acid was also reported in relatively high concentration in 'Mayonnaise' (mean LB = UB = 2,700 mg/kg) indicating, presumably, the use of rapeseed oil or mustard during its preparation.

Erucic acid could not be quantified in milk or many different types of cheese. However, for some particular cheese varieties prepared/preserved using oil and/or spices (Gouda, Feta, Trappist), the presence of erucic acid was reported (mean LB ranging from 60 to 200 mg/kg).

The only reported levels of erucic acid for the food group 'Legumes, nuts and oilseeds' corresponded to samples of 'Rape seeds' and 'Peanuts'. Since peanuts do not naturally contain erucic acid (Carrín and Carelli, 2010), the levels are assumed to originate from oil used for roasting the nuts.

It is also important to note the presence of erucic acid in 'Fine bakery wares' which indicates the common use of rapeseed oil in the preparation of these products. For 'Pastries and cakes', erucic acid was quantified in half of the samples (mean 240/290 mg/kg (LB/UB)) with a 95th percentile concentration of 1,100 mg/kg (LB = UB). The average levels of erucic acid were even higher in 'Biscuits' (mean 270/390 mg/kg (LB/UB)) so it was the 95th percentile concentration (1,800 mg/kg LB = UB). However, the prevalence of erucic acid in 'Biscuits' was lower than in 'Fine bakery wares' since only in about 25% of the samples erucic acid was quantified.

Erucic acid in 'Starchy roots and tubers' should be in general non-existent or negligible; the quantified samples reported in Appendix C, Table C.5 correspond to processed foods (e.g. French fries, potato croquettes), where the erucic acid originates from the oil used during cooking.

No erucic acid was quantified in the few samples of 'Vegetables and vegetable products (including fungi)' except in one sample of mustard sprouts for which an erucic acid concentration of 179,000 mg/kg was reported. This is in accordance with the high levels of erucic acid for mustard seeds mentioned in Section 3.1.1.1.

Within the food group 'Sugar and confectionary' particularly high levels of erucic acid were quantified in some samples of chocolate spread codified as 'Chocolate, cream' (170/250 mg/kg (LB/UB)). In total, twelve samples out of thirty-two reported the presence of erucic acid. The most plausible reason

[30] Adulteration with 5% rapeseed oil to arrive to 2,700 mg/kg it would imply rapeseed oil with erucic acid content above 50,000 mg/kg (HEAR).

for the levels of erucic acid reported in the samples of 'Chocolate, cream' should be the oil used in its preparation. Among the samples reported as 'Products for special nutritional use' the highest levels of erucic acid were quantified in samples of 'Supplements containing special fatty acids (e.g. omega-3, essential fatty acids)' with mean values of 4,200/4,300 mg/kg (LB/UB) (n = 26) and 'Unspecified dietary supplements' with mean values of 12,200/12,400 mg/kg (LB/UB) (n = 22).

A total of 810 samples were reported for the food group 'Food for infants and small children'. They were mainly contained in four groups, 'Infant formulae, powder' (n = 218), 'Follow-on formulae, powder' (n = 191), 'Cereal-based food for infants and young children' (n = 166) and 'Ready-to-eat meal for infants and young children' (n = 156). Overall, relatively low levels of erucic acid were reported for these samples. The highest mean values were reported for 'Infant formulae, powder' (220/290 mg/kg (LB/UB)) and the lowest for 'Ready-to-eat meal for infants and young children' (77/86 mg/kg (LB/UB)).

The CONTAM Panel noticed that erucic acid can be present in different food additives derived from fatty acids. Among them, ammonium phosphatides (E 442), obtained from partially hardened rapeseed oil (FAO/WHO, 2006) could be mentioned, but also mono- and diglycerides and polyglycerol esters of fatty acids (E 470a, E 470b, E 471, E 472a, E 472b, E 472c, E 472d, E 472e, E 472f, E 475, E 477, E 479b), which are used as emulsifiers in many different food commodities. However, the contribution to the presence of erucic acid from the use of these additives is expected to be minor as compared to that coming from the presence of rapeseed oil as ingredient.

As shown in Figure 2, the samples contained in the dataset used to estimate exposure were collected in 13 different European countries, most of them in Germany (4,822 samples). For more than 5,000 samples, the sampling country was reported as 'European Union'; they were mainly reported by FEDIOL but also by SNE. These samples mainly corresponded to rapeseed oil (99%). The samples were collected between 2000 and 2015, with half of the samples collected in 2014 (Figure 3).

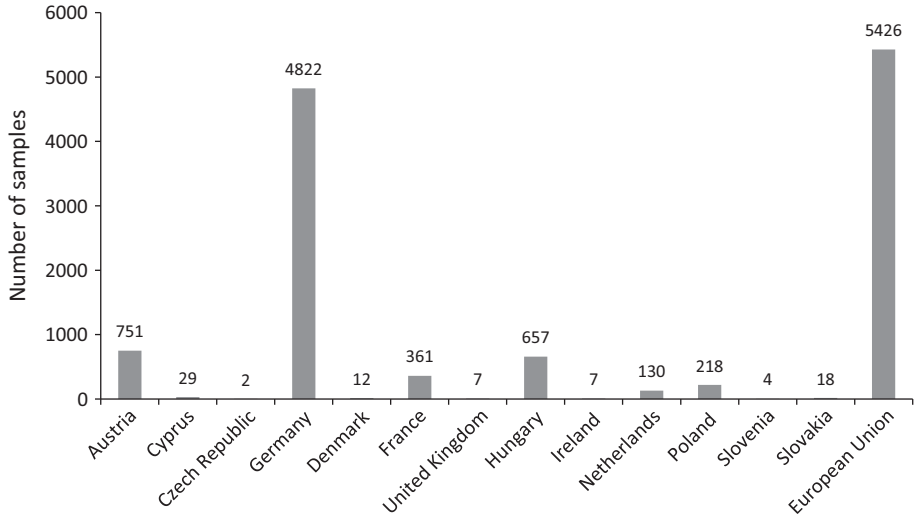


Figure 2: Distribution of food samples analysed for erucic acid across different European countries

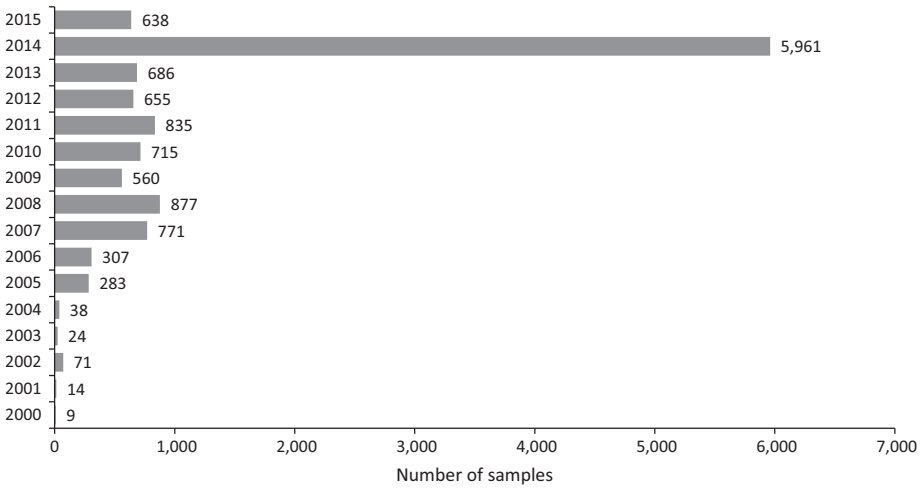


Figure 3: Distribution of food samples analysed for erucic acid over the sampling years

Analytical methods

Only limited information was reported on the analytical methods used to analyse erucic acid. For around 30% of the cases no information was provided. In 10% of the samples only the chromatographic technique used in the analysis was reported (GC) without further information on the method of detection. For the remaining samples (60%) GC-FID was used.

Among the samples reported as being analysed by GC-FID a very wide range of LOQs was noted. The lowest LOQ was reported for the analysis of unspecified savoury sauces (0.042 mg/kg) while the highest was reported for the analysis of some samples of rapeseed oil (20,000 mg/kg). More detailed information on the reported LOQs in the different food groups (at FoodEx level 1) is shown in Table 4.

Table 4: Range of limits of quantification in food samples (FoodEx 1 level) reported as analysed by gas chromatography-flame ionisation detection (6,958 samples) from the final dataset (n = 12,444) used to estimate dietary exposure assessment

FoodEx level 1	Reported limits of quantification (mg/kg; whole weight)		
	N	Minimum	Maximum
Grains and grain-based products	26	1.4	1,000
Vegetables and vegetable products (including fungi)	22	500	1,000
Legumes, nuts and oilseeds	3	100	5,000
Fish and other seafood	44	3,333	3,333
Milk and dairy products	338	2.8	1,000
Eggs and egg products	21	100	1,000
Sugar and confectionary	193	500	1,000
Animal and vegetable fats and oils	5,753	2	20,000
Herbs, spices and condiments	17	0.042	1,000
Food for infants and small children	517	0.24	500
Products for special nutritional use	7	4.8	1,000
Snacks, desserts, and other foods	17	1,000	1,000

N: number.

3.1.2.2. Feed

A total of 270 feed samples were initially reported. One sample of 'Rape seed', which was reported on a fat weight basis but without further information on its fat content, was excluded. In addition, six samples wrongly reported as food were re-codified as feed, leading to a final number of 275 feed samples. Table 5 shows the feed samples classified according to the catalogue of feed materials

described in Commission Regulation 68/2013.[31] Apart from the 193 samples of rapeseed oil, a very limited number of feed samples was available.

Erucic acid concentrations of 193 samples of rapeseed oil were provided, with mean values of 1,300/4,200 mg/kg (LB-UB), respectively. Oil extraction from seeds involves a number of processes. The first is a physical process, resulting in the production of rapeseed 'cake', with oil contents typically ~ 10%. This may be used as livestock feeds, particularly in organic production systems. However, most of the cake undergoes a second process involving solvent extraction, resulting in a 'meal' with oil contents nearer to 3–5%.[31] Since erucic acid is extracted in the oil, it follows that levels are likely to be higher in the cakes than the meals. A number of samples (n = 28) were reported as either 'rapeseed expeller' or 'rapeseed meal'. However, as these samples contained a rather high amount of fat (~ 10%) they were all considered as 'rapeseed expeller', which usually has fat contents similar to those reported. Average levels of erucic acid in the samples of rapeseed expeller were 460/470 mg/kg (LB/UB). In addition, data on 21 samples of sunflower oil were provided. Sunflower oil is not normally used in feed for livestock, and since all of these were left-censored, they were not included in estimates of exposure.

Table 5: Mean and 95th percentile concentrations (mg/kg) in feed samples classified according to the Catalogue of feed materials specified in Commission Regulation (EU) No 68/2013[32]

					Mean (mg/kg)[(b)]		95th percentile (mg/kg)[(a),(b)]	
			N	% LC	LB	UB	LB	UB
Cereal grains, their products and by-products	Maize	Maize germ	1	100	0	20	–	–
Miscellaneous	Fatty acids	Fatty acids	2	0	626	626	–	–
Oil seeds, oil fruits, and products derived thereof	Rape seed	Rapeseed, expeller	28	11	456	467	–	–
	Toasted soy (beans)	Soy (bean) expeller	1	100	0	1,000	–	–
		Soy beans, extruded	1	0	180	180	–	–
	Sunflower seed	Sunflower seed	1	100	0	20	–	–
	Camelina seed	Camelina, expeller	1	0	3,670	3,670	–	–
	Vegetable oil and fat	Rapeseed oil	193	83	1,326	4,165	9,000	9,000
		Sunflower oil	21	100	0	1,190	–	–
		Borage oil	2	0	26,384	26,384	–	–
		Unspecified vegetable oil	1	0	1,195	1,195	–	–
Other seeds and fruits, and products derived thereof	Grape pips	Grape pips meal	1	100	0	20	–	–
Land animal products and products derived thereof	Animal fat (Feed)	Animal fat (Feed)	12	0	1,231	1,231	–	–

[31] Source: Animal Feed Resources Information System (www.feedipedia.org).
[32] Commission Regulation (EU) No 68/2013 of 16 January 2013 on the Catalogue of feed materials Text with EEA relevance. OJ L 29, 16.1.2013, p. 1.

			N	% LC	Mean (mg/kg)[b]		95th percentile (mg/kg)[a],[b]	
					LB	UB	LB	UB
Compound feed	Compound feed, unspecified	Compound feed, unspecified	2	0	1,500	1,500	–	–
	Complete feed	Fattening chickens (broilers)/ complete feed	3	100	0	20	–	–
		Pet food, dogs/ complete feed	3	0	429	429	–	–
		Pet food, cats/ complete feed	1	0	1,000	1,000	–	–
		Piglets (weaning diets)/complete feed	1	0	144	144	–	–

N: number of samples; LB: lower bound; LC: left-censored; UB: upper bound.
(a): The 95th percentile with less than 60 observations may not be statistically robust (EFSA, 2011b). Those estimates were not included in this table.
(b): Values were rounded to the nearest whole number (0 decimal places).

In most of the samples (n = 201), reported by FEDIOL, the country of sampling was only specified as 'European Union' without further information. Among the samples for which information was reported, the main sampling countries were Poland (n = 47) and Denmark (n = 12). With regard to the sampling year, samples were collected between 2003 and 2015, with the highest prevalence for the years 2014 (n = 68) and 2015 (n = 150).

GC with FID detection was the selected method for the analysis of most of the feed samples (n = 259). The lowest LOQ was reported for the analysis of rape seeds, 39 mg/kg whole weight, while the highest LOQ was reported for the analysis of rapeseed oil (5,000 mg/kg whole weight).

3.1.3. Food and feed processing

Erucic acid is most commonly found in seeds of the Brassicaceae family, which are widely grown as sources of oil both for human consumption (e.g. oilseed rape) and industrial purposes (e.g. HEAR or crambe). The seeds are initially physically pressed to remove the oil, and then in most cases, the 'expeller cake' is processed further by solvent extraction, leaving the 'meal'. Most of the erucic acid is extracted with the oil. While some remains in the rapeseed expeller (after crushing) and even less in the rapeseed meal (after solvent extraction), levels are significantly lower than in the original seed.

After extraction and refining, some uses of the oil require its chemical transformation to acquire the plasticity demanded by the margarine and shortening industries. Such transformation can be achieved by hydrogenation, which consists in addition of hydrogen to the oil in the presence of a catalyst, a process that 'removes' double bonds and subsequently reduces the degree of unsaturation of the oil (Farr, 2005). The reduction of the degree of unsaturation not only confers plasticity to the oil, but also increases its oxidative stability, as double bonds in the fatty acid chain are the main oxidation centres in the oil. This makes partially hydrogenated oils particularly suitable for high temperature uses such as deep frying (Rossell, 2001). During partial hydrogenation *trans* isomers of fatty acids can be formed. For example, margarines are reported to contain from 3 to 26 g/100 of *trans* fatty acids (Tarrago-Trani et al., 2006). *Trans* fatty acids can be also formed during the refining of the oil, particularly in the deodorisation step, although at lower scale than in the hydrogenation process (Martin et al., 2007). The effect of hydrogenation on the fatty acid profile of the oil depends on the degree of hydrogenation and the conditions of the process. In fish oil with high 22:1 content (e.g. herring oil), conventional partial hydrogenation reduces only slightly the total content of 22:1 fatty acids but about 50% of them change from *cis* to *trans* configuration. In addition to geometrical isomers, partial hydrogenation results in the formation of positional isomers (Opstvedt et al., 1990), for example 22:1 n-13 and 22:1 n-15, which are difficult to separate from 22:1 n-11 in chromatographic analysis of fatty acid methyl esters (Shimizu and Ando, 2012).

3.2. Dietary exposure assessment

3.2.1. Dietary exposure assessment of erucic acid in humans

3.2.1.1. Previously reported exposure assessments in humans

FSANZ estimated dietary exposure to erucic acid from canola oil (FSANZ, 2003). Using the highest reported level of erucic acid (1.6%), the mean intake for consumers only was 124 mg/day and for high consumers 348 mg/day, corresponding to 1.8 and 5.0 mg/kg body weight (bw) per day assuming a body weight of 70 kg.

Based on a 24-h dietary recall, dietary intakes of erucic acid were estimated during the National Health and Nutrition Examination Survey (NHANES) 1999–2000 survey (Ervin et al., 2004). The mean dietary intake was 40 mg/day (median 10 mg/day) across the different age classes. The mean intake was 20 mg/day for children under 6 years, corresponding to 1.7 mg/kg bw per day using the default body weight of 12 kg for toddlers (EFSA Scientific Committee, 2012). For adults (20–59 years), the mean intake was 50 mg per day, corresponding to 0.7 mg/kg bw per day assuming a body weight of 70 kg.

Udipi et al. (2006) estimated the fatty acid intakes of healthy adult males (n = 25/region) from three regions in India using dietary records, food frequency questionnaires and chemical analysis of the diet. The mean erucic acid intake was reported only for the region West Bengal and was 17.3 ± 8.3% of the total fat intake. The authors reported that this high erucic acid intake was due to the high mustard oil consumption. By using the total fat intake of 70.9 ± 21.3 g/day reported for this region, the CONTAM Panel calculated an erucic acid exposure of 12.3 g/day or 180 mg/kg bw per day for a 70 kg adult.

3.2.1.2. Current dietary exposure assessment in humans

Chronic dietary exposure was estimated across Europe following the methodology described in Section 2.2.1.1. Before linking consumption and occurrence data, some adjustments of the consumption data were done to reduce uncertainty and reach more accurate exposure estimates. Among these adjustments, it is worth mentioning the use of a factor of 0.125 to convert the reported consumption data on both liquid 'Infant formula' and liquid 'Follow-on formula' into powder as only occurrence data on the latter form were available (see Appendix C, Table C.5). Likewise, factors of 0.25 (when reconstituted with water) and 0.15 (when reconstituted with milk) were applied to the food group 'Cereal-based food for infants and young children' when the eating occasions were reported as consumed (liquid) since the occurrence data also referred to the analysis of the powder form.

When no plausible reasons exist that may justify the presence of erucic acid, samples that belonged to one particular food group and that were all reported as left-censored data were not used for dietary exposure estimations to avoid unwanted bias in the UB estimations. These samples mainly belonged to food groups such as 'Drinking water', 'Fruit and fruit products', 'Eggs and egg products' and 'Non-alcoholic beverages' among others (Appendix C, Table C.5).

Table 6 shows summary statistics of the chronic dietary exposure assessment to erucic acid using the available occurrence data. Detailed mean and 95th percentile dietary exposure estimates calculated for each of the 35 dietary surveys are presented in Appendix C, Table C.6.

Table 6: Summary statistics of chronic dietary exposure assessments to erucic acid across European dietary surveys (mg/kg bw per day)

Age class[a]	N	Mean dietary exposure (mg/kg bw per day)					
		LB[c]			UB[c]		
		Min	Median	Max	Min	Median	Max
Infants	6	0.7	1.8	2.8	1.7	2.6	3.7
Toddlers	10	1.2	2.0	2.8	1.7	2.8	4.4
Other children	18	1.0	1.7	2.4	1.5	2.5	3.7
Adolescents	17	0.5	1.0	1.6	0.8	1.3	2.2
Adults	17	0.3	1.0	1.5	0.6	1.3	1.9
Elderly	14	0.3	1.0	1.4	0.5	1.3	1.8
Very elderly	12	0.4	1.0	1.3	0.6	1.3	1.6

Age class[a]	N	95th percentile dietary exposure[b] (mg/kg bw per day)					
		LB[c]			UB[c]		
		Min	Median	Max	Min	Median	Max
Infants	5	1.7	4.1	5.8	4.5	5.7	7.4
Toddlers	7	3.2	4.1	4.4	4.9	5.5	6.5
Other children	18	2.1	4.0	5.3	3.1	4.9	9.5
Adolescents	17	1.2	2.4	4.8	1.7	2.9	5.4
Adults	17	0.9	2.1	3.8	1.3	2.6	4.3
Elderly	14	0.7	2.4	3.9	1.1	2.9	4.2
Very elderly	9	1.1	2.7	3.2	1.4	3.1	3.5

bw: body weight; LB: lower bound; Max: maximum; Min: minimum; n: number of surveys; UB: upper bound.
(a): Section 2.1.2.1 describes the age range within each age class.
(b): The 95th percentile estimates obtained on dietary surveys/age classes with less than 60 observations may not be statistically robust (EFSA, 2011b). Those estimates were not included in this table.
(c): Estimates were rounded to one decimal place.

Highest chronic dietary exposure was estimated in the youngest population. Concerning mean dietary exposure, the highest estimate at the LB corresponded to the age classes 'Infants' and 'Toddlers' with a maximum value of 2.8 mg/kg bw per day, while at the UB the maximum estimate was observed in the age class 'Toddlers' (4.4 mg/kg bw per day). In the highly exposed population, referring to the 95th percentile of the distribution of the exposure for each dietary survey and age class, the highest estimates were in 'Infants' with values of 5.8/7.4 mg/kg bw per day (LB/UB) and 'Other Children' (5.3/9.5 mg/kg bw per day (LB/UB)). Dietary exposure in specific groups of the population, namely 'Pregnant women' and 'Lactating women', were within the range of exposure estimates in the adult population.

Detailed contribution of the different food categories at FoodEx level 1 and grouped by age classes is shown in Appendix C, Table C.7. Estimations of exposure at the middle bound (MB) were used to explain the contribution of the different food commodities.

Overall, the main contributor to dietary exposure to erucic acid was the food group 'Fine bakery wares', more precisely 'Pastries and cakes' and 'Biscuits (cookies)'. This food group was particularly important in 'Toddlers' and 'Other children', although it was also a main contributor to the dietary exposure in the adult population. At the MB, the contribution of 'Fine bakery wares' in 'Toddlers' represented up to 39% of the total exposure (median = 21%), and in 'Other children' contributed up to 48% to the total exposure (median = 27%). Since the levels of erucic acid in 'Fine bakery wares' ('Pastries and cakes' and 'Biscuits (cookies)') are not that high (240/390 mg/kg, LB/UB), its relevant contribution is probably mainly driven by the relatively high consumption of this heterogeneous food category (e.g. croissants, doughnuts, cakes, muffins, waffles, biscuits, cookies, etc.). The reported values on erucic acid for 'Pastries and cakes' and 'Biscuits' (see Appendix C, Table C.5) show that, as commented above, the use of rapeseed oil in the elaboration of these food commodities seems to be rather common practice in industry.

Two other food groups that were also relatively important contributors to the dietary exposure to erucic acid across all age classes (excluding 'Infants') were 'Potatoes and potato products' and 'Margarine and similar products', processed food commodities where rapeseed oil seems to be used in their preparation. In both cases, the contribution at the MB was greater than 20% in several population groups, with 'Potatoes and potato products' reaching up to 29% and 'Margarine and similar products' up to 27% of the total exposure to erucic acid.

In the age class 'Infants', 'Food for infants and small children' (FoodEx level 1) was the main contributor to the exposure. Among the different types of food for infants, the food group 'Ready-to-eat meal for infants and young children' was the most important contributor in the dietary survey with the highest exposure. The contribution of this food group went up to 52% at the MB scenario (range 19–52%) among the dietary surveys for 'Infants'. Other food groups such as 'Cereal-based food for infants and young children', 'Follow-on formulae, powder' and 'Infant formulae, powder' also had an important contribution in different dietary surveys for 'Infants'.

For the adult population, the food group 'Condiments' was the main contributor to the exposure in a few dietary surveys, representing in some cases half of the total contribution (MB approach). The high contribution of 'Condiments' was driven by the consumption of 'Mustard, mild' that although

consumed in low amounts contains very high levels of erucic acid (~ 14 g/kg). The contribution of 'Fish meat' to the total exposure to erucic acid was also important in some adult populations in different dietary surveys, with contributions up to 41% of the total exposure (MB scenario).

The contribution of rapeseed oil to the total dietary exposure to erucic acid was, in most of the cases, limited. However, in few dietary surveys, the consumption of rapeseed oil played an important role reaching average contributions (MB scenario) up to 63%, with an average contribution of 39% in the dietary survey with the highest exposure estimate.

Specific exposure scenarios

Considering the relatively low levels of erucic acid in 'Human milk' (Table 3), the CONTAM Panel concluded that a specific scenario to evaluate the exposure to erucic acid via the consumption of human milk was not pertinent. Likewise, considering the occurrence values described in Section 3.1.2.1 and the main contributors to dietary exposure to erucic acid, a specific scenario for vegetarians was considered not necessary.

Specific exposure scenario for composite foods and custard

Potential dietary exposure to erucic acid via the consumption of composite food (ready-to-eat-food) was also evaluated for adults and toddlers (Table 7). To estimate exposure, the occurrence values reported to EFSA for different types of composite food (see Appendix C, Table C.4) were combined with chronic consumption of these commodities (consumers only) obtained from the Comprehensive database. The consumption of 'Pasta, cooked' used in the estimations refers to the consumption value obtained from the Comprehensive database multiplied by a factor of 2.5 to convert the raw amount into cooked.

Similarly, the consumption data were also used to estimate the potential exposure to erucic acid from the consumption of custard with high levels of erucic acid. As mentioned in Section 3.1.2.1, erucic acid is not expected to be found in samples of custard; however, as two samples were reported with relatively high levels of erucic acid (1,010 mg/kg, LB = UB) the CONTAM Panel decided to create a scenario to consider the potential exposure to erucic acid through the consumption of this dessert.

Table 7: Dietary exposure to erucic acid from consumption of specific composite foods and custard combining reported occurrence values for these foods (Appendix C, Table C.4 and Section 3.1.2.1) with chronic consumption data (consumers only)

Mean dietary exposure[a] (mg/kg bw per day)						
	Toddlers			Adults		
	% consumers	Exposure LB	Exposure UB	% consumers	Exposure LB	Exposure UB
Pasta, cooked	25–94	2.9–23.7	2.9–23.7	2–90	0.8–11.2	0.8–11.2
Meat-based meals	2–72	0.7–1.2	0.7–1.2	0.1–91	0.1–0.8	0.1–0.8
Ready-to-eat soups	0.5–64	0.02–0.5	0.1–1.5	1–70	0.002–0.1	0.01–0.2
Prepared salads	0.3–14	1.1–2.6	1.1–2.6	0.1–80	0.4–3.2	0.4–3.2
Custard	0.4–37	0.7–5.6	0.7–5.6	0.2–32	0.1–3.2	0.1–3.2
95th percentile dietary exposure[b] (mg/kg bw per day)						
	Toddlers			Adults		
	% consumers	Exposure LB	Exposure UB	% consumers	Exposure LB	Exposure UB
Pasta, cooked	25–94	7.5–41.9	7.5–41.9	2–90	2.4–24.3	2.4–24.3
Meat-based meals	2–72	1.8–2.0	1.9–2.1	0.1–91	0.3–1.2	0.3–1.3
Ready-to-eat soups	0.5–64	0.1–0.2	0.3–0.8	1–70	0.01–0.2	0.02–0.5
Prepared salads	0.3–14	–	–	0.1–80	1.1–7.9	1.1–7.9
Custard	0.4–37	7.4–14.1	–	0.2–32	0.2–3.3	0.2–3.3

bw: body weight; LB: lower bound; UB: upper bound.
(a): Range of mean dietary exposure (mg/kg bw per day) across all dietary surveys.
(b): Range of 95th dietary exposure (mg/kg bw per day) across all dietary surveys. Only dietary surveys with more than 60 consumers were considered.

Table 7 shows that the only consumption of prepared dishes of pasta at the reported mean levels of erucic acid (2,700 mg/kg) might result in a high exposure to this substance as compared to the levels reported in Table 6 (having in mind that estimates in Table 7 refer to consumers only). Both maximum mean exposure (UB) and maximum 95th percentile dietary exposure (UB) via the consumption of pasta in consumers only were around 6-fold higher than the maximum exposure estimates in 'Toddlers' and 'Adults' considering the whole diet.

The origin of the erucic acid in the samples of pasta is uncertain, although it seems evident that it is not from the pasta itself but more from the rapeseed oil and/or different spices that may have been used to condiment the pasta. It is worth commenting that these results should be interpreted very cautiously since they are based on only three samples. Similar interpretation should be done for the exposure estimations derived from the consumption of 'Custard' (n = 2) and 'Prepared salads' (n = 3).

As commented in Section 3.1.1.1, several samples of 'Pickles' with very high levels of erucic acid have been reported in RASFF in recent years. Although pickles seem to be rarely consumed in Europe (around 1,000 consumers for 'Pickles and chutneys' in the Comprehensive database, 0.1–14% consumers across dietary surveys), it is important to note that due to the high levels of erucic acid reported, even the consumption of small amounts of this food commodity may result in very high exposure to erucic acid. If we considered an average content of 15 g/kg of erucic acid (whole weight) extracted from RASFF notifications, and average and 95th chronic consumption for 'Pickles and chutneys' from the Comprehensive database (consumers only), the range of chronic exposure to erucic acid via the consumption of pickles in the adult population would be 0.9–7.8 mg/kg bw per day among the mean consumers and 2.9–7.8 mg/kg bw per day[33] for the high consumers (only four dietary surveys considered with at least 60 consumers). These exposure estimations should be carefully interpreted since there are doubts on whether the RASFF notifications refer to the oil used to preserve the pickle or to the pickle itself, and due to the fact that the consumption data do not distinguish between pickles preserved in oil or in brine/vinegar solutions.

3.2.1.3. Non-dietary exposure

Additional exposure may occur from the commercial use of erucic acid in cosmetics, lubricants, surfactants, textiles, polymers and inks (Töpfer and Martini, 1998; Murphy, 2012). However, absorption via the skin is not known and the contribution to the total exposure via this route is not known.

3.2.2. Dietary exposure assessment of erucic acid in animals

3.2.2.1. Previously reported exposure assessments in animals

The CONTAM Panel has not identified any previous assessments of exposure to erucic acid by farm livestock or companion animals.

3.2.2.2. Current exposure assessment in animals

The main source of exposure to erucic acid is from the seeds of oilseed rape (*Brassica napus*). Seeds and meals from other Brassicaceae crops may also be used, but generally in niche animal feeds, and no data are available on their use. In the absence of sufficient data on levels of erucic acid in other oilseeds and their meals, only data for rapeseed meal and rapeseed oil have been used to estimate exposure to erucic acid. A number of plants of the Brassicaceae family, including swedes, turnips, kale and forage rape, are grown as feeds for ruminants, but are fed whole and before the plant matures and seed development commences. Since erucic acid is only found in the seeds of *Brassica* plants, these crops do not represent a source of exposure to erucic acid.

While some whole seeds are fed to livestock (generally restricted to pig and some poultry diets), most are subjected to oil extraction with the resulting cake or meal used as feed. Within the EU, some 6.8 million tonnes of rapeseed meals were used in the manufacture of compound feeds in 2012/13, of which 5 million tonnes originated in the EU.[34] In addition, rapeseed meals may be fed directly to livestock on farms as part of their daily ration, although there are no data on the amounts used in this way.

Rapeseed cakes and meals are important feed materials in diets for all livestock in the EU. They are principally used as a source of protein, and are included in the diets of all farm animals. The amounts fed will be determined by a number of factors, including the nutritional requirements of the animals

[33] The maximum mean consumption across dietary surveys was equal to the maximum 95th chronic consumption resulting in the same exposure estimate.
[34] Source: FEFAC statistics (www.fefac.eu).

and the cost of the cakes/meals relative to other sources of protein. Vegetable oils, including rapeseed oil, are also important ingredients of livestock diets, predominantly because of their high energy content. Levels of inclusion are variable, according to the nutritional needs of the animal, but generally do not exceed about 5% of the total ration. Rapeseed oil is not normally the sole vegetable oil used, but will be part of a blend of oils.

In the absence of data on the amounts of rape seeds and rapeseed products consumed on a daily basis, the CONTAM Panel has used published recommended maximum inclusion levels for rapeseed meal and oil for each of the different categories of livestock (Ewing, 1997; Canola Council of Canada, 2015). These recommended levels have been applied to the mean lower bound (LB) and upper bound (UB) levels reported for rapeseed oil and rapeseed meal (Table 5) to obtain levels of erucic acid in the diets and estimated exposures. These are given in Tables 8–10 below. Insufficient data on levels of erucic acid in rapeseed meal or cake were available to allow P95 estimates of exposure to be made. Details of feed intakes and diet compositions used to estimate exposure are given in Appendix D.

Ruminant and horses

Rapeseed meal is widely used in the diets of ruminants, and in this Opinion, an inclusion rate of 25% in the compound feed has been assumed (20% for lactating sheep and lactating and fattening goats) (Ewing, 1997). The use of rapeseed oil, regardless of the erucic acid level, is limited due to potential adverse effects on rumen fermentation, although it may be present in blends of vegetable oils used in compound feeds. Estimates of exposure have assumed a maximum inclusion rate in the compound feed of 2%.

Table 8: Estimated mean lower bound and upper bound diet concentration and daily exposure by ruminants and horses to erucic acid

	Diet concentration (mg/kg)	Intake (mg/day)	Intake (mg/kg bw per day)
Dairy: high yielding			
LB	56	1,164	1.8
UB	80	1,656	2.6
Beef: fattening			
LB	21	202	0.51
UB	30	288	0.72
Sheep: lactating			
LB	59	165	2.8
UB	88	247	4.1
Goats: lactating			
LB	88	300	5.0
UB	132	451	7.5
Goats: fattening			
LB	47	71	1.8
UB	71	106	2.7
Horses			
LB	48	427	0.95
UB	77	690	1.5

bw: body weight; LB: lower bound; UB: upper bound.

Pigs, poultry, salmonids and rabbits

Levels of rapeseed meal in diets of pigs, poultry, salmonids and rabbits vary from 5% to 30%, depending on the species and/or age of the animal. Levels of rapeseed oil also vary; in practice lower amounts are used in pig diets because of the effect on the level and composition of body fat. For poultry, it is common to add 1.0–1.5% rapeseed oil to the diet (Canola Council of Canada, 2015). Full-fat rape seed, after particle size reduction (rolling) is an important protein and energy ingredient in broiler diets in a number of EU countries, but in the absence of data on erucic acid content of full-fat rape seed this has not been included in estimates of exposure.

Table 9: Estimated mean lower bound and upper bound diet concentration and daily exposure by pigs, poultry, salmonids and rabbits to erucic acid

	Diet concentration (mg/kg)	Intake (mg/day)	Intake (mg/kg bw per day)
Pig starter			
LB	85	85.4	4.3
UB	172	172	8.6
Pig finisher			
LB	131	393	3.9
UB	218	655	6.6
Lactating sow			
LB	131	786	3.9
UB	218	1,310	6.6
Chickens for fattening			
LB	157	18.8	9.4
UB	203	24.3	12
Laying hens			
LB	111	13.3	6.7
UB	156	18.7	9.4
Turkeys for fattening			
LB	131	52.4	4.4
UB	218	87.3	7.3
Ducks for fattening			
LB	108	15.1	5.1
UB	195	27.3	9.1
Salmonids			
LB	158	6.30	3.2
UB	302	12.1	6.
Rabbits			
LB	85	12.8	6.4
UB	172	25.7	13

bw: body weight; LB: lower bound; UB: upper bound.

Companion animals (cats and dogs)

Information provided to EFSA by the European Pet Food Industry Federation (FEDIAF) indicated that meal, rapeseed oil and meals and oils from other Brassicaceae crops are not common constituents of cat and dog foods (FEDIAF, Personal communication by email, May 2016). Therefore, no exposure assessments have been undertaken for these animals.

3.3. Hazard identification and characterisation

3.3.1. Toxicokinetics

3.3.1.1. Absorption of erucic acid

Most studies on the absorption of erucic acid have been conducted using oils with a high content of erucic acid, such as rapeseed or mustard oil. In order to take into account both the lipase-catalysed hydrolysis of the triacylglycerols in the intestine and the passage of the released erucic acid through the intestinal mucosa, the apparent coefficient of digestibility was calculated from the amount of fat ingested minus the amount excreted with the faeces, and expressed as percentage of the ingested fat. In general, the digestibility of triacylglycerols depends primarily on the type of acyl groups (fatty acids) and their location in the triacylglycerol molecule. Fatty acids located at position 2 of the triacylglycerol

are faster released than fatty acids at positions 1 and 3. Erucic acid is predominantly located at positions 1 and 3.

The apparent digestibility coefficients of rapeseed oil with a high content of erucic acid has been determined for several species including humans and found to vary between 58% and 100%, depending on the species and also somewhat on the amount of oil in the diet (Table 10). In these studies, only the digestibility of total rapeseed oil but not that of erucic acid has been determined. High values of near 100% for the digestibility of rapeseed oil have consistently been found for humans. Digestibility appears to be also very high in swine and dogs but somewhat lower in rodents, although strain differences exist, e.g. between Sprague–Dawley and Wistar rats (Table 10).

Table 10: Digestibility of rapeseed oil in humans and various animal species receiving diets containing high erucic acid rapeseed oil (in human studies: 2.5–10%, in animal studies: 15–25%)

Species	Age, gender, strain	Digestibility (%)	Reference
Humans	Adults, gender not specified	99	Deuel et al. (1949)
	Male and female adults	96	Vaisey et al. (1973)
Dogs	Weaned, gender and strain not specified	94	Crampton et al. (1960)
Swine	Weaned, gender and strain not specified	78	Crampton et al. (1960)
	Adult Large White Yorkshire, gender not specified	94–100	Paloheimo and Jahkola (1959)
Guinea pigs	Weaned, gender and strain not specified	72	Crampton et al. (1960)
	Adult males, strain not specified	61	Carroll (1957)
Lambs	3-day-old, gender not specified, cross-bred	62	Walker and Stokes (1970)
Rabbits	Adult males, strain not specified	58	Carroll (1957)
Rats	Adult females, strain not specified	77–82	Deuel et al. (1948)
	Adult males, Sprague–Dawley	58	Carroll (1957)
	Adult males, Sprague–Dawley	65	Beare et al. (1960)
	Adult males, Wistar	83	Beare et al. (1960)

Rocquelin and Leclerc (1969) compared the digestibilities of rapeseed oil with a high (44.7%) and a 'nearly zero content' of erucic acid (1.9%), and peanut oil in Wistar rats fed diets containing 15% by weight of one of the oils up to 9 days. There was no significant difference in feed consumption between the groups, but faecal excretion of dry material was more enhanced in rats fed with diets containing high erucic acid levels. A lower digestibility (81%) was noted for rapeseed oil with a high content of erucic acid compared to the other two fats (92–95%). Erucic acid and eicosenoic acid, representing 55% of the total fatty acid content of high erucic acid rapeseed oil, appear to be responsible for the lower digestibility of the oil, as they represent 85% of the faecal fatty acids.

The coefficient of digestibility was studied in young male Sprague–Dawley rats for free erucic acid in comparison to some of its esters. Free erucic acid had a digestibility of 53% while methyl erucate had 62% and ethyl erucate 59% (Carroll, 1958). In another study, free erucic acid had a digestibility of 48% and glycerol trierucate 63% (Carroll and Richards, 1958). The coefficient of digestibility was shown to be slightly affected by a number of other factors, e.g. the level of protein and calcium in the diet, and the age of the animals (Carroll and Richards, 1958).

Sergiel and Gabucci (1980) studied the digestibility and faecal excretion patterns in Wistar rats of erucic and brassidic acid esterified in different triacylglycerol structures. Rapeseed and peanut oils were used as controls. Rats were fed diets containing 15% by weight of one of the oils for up to 11 days. Digestibility of glycerol trierucate or interesterified glycerol trierucate was similar to that of peanut oil but higher than that of rapeseed oil, whereas digestibility of glycerol tribrassidate and interesterified glycerol tribrassidate was lower. The high amount of glycerol-2-monoerucate, resulting from diets containing glycerol trierucate, explained the better digestibility of erucic acid as compared to that of rapeseed oil which had a very small erucic acid content on the 2-position. Glycerol tribrassidate had a poor digestibility because its hydrolysis by pancreatic lipase was delayed in the intestinal lumen.

After oral intake of rapeseed oil, the triacylglycerols in human lymphatic fat exhibited the same percentage of erucic acid as in the rapeseed oil (Fernandes et al., 1955), while the percentage of

erucic acid in rat intestinal lymph triacylglycerols was about 70% of that of the dietary oil (Caselli et al., 1979), consistent with the higher digestibility in humans (see above).

A radiotracer study on the absorption of free erucic acid in young adult rats has been conducted by Carroll (1962). After oral dosing of 2-^{14}C-erucic acid, about 20% and 5% of the administered radioactivity was still in the intestinal tract after 6.5 and 24 h, respectively. Nearly the same values were found for palmitic acid (16:0) and nervonic acid (24:1 n-9). However, palmitic acid produced more respiratory carbon dioxide during the first 6 h than erucic and nervonic acid, suggesting differences in the rate of β-oxidation.

3.3.1.2. Distribution of erucic acid

Like other long-chain fatty acids, free erucic acid is assumed to be transported in the blood mainly bound to serum albumin. A recent study has elucidated the interaction of erucic acid with bovine serum albumin (Shu et al., 2015). Using multiple spectroscopic methods and molecular docking techniques, it was shown that erucic acid binds to one site in the IIA subdomain, mainly through hydrophobic but also hydrogen bonding.

Wagner et al. (1958) fed a diet containing rapeseed oil with 42% erucic acid to adult male rats for 6 months, and subsequently determined the content of erucic acid in the fractions of neutral lipids and phospholipids of various organs (heart, liver, kidneys, lung, spleen, intestine, bones and carcass). The rat strain and concentration of rapeseed oil in the diet were not given. The percentage of erucic acid among total fatty acids varied between different organs, being in neutral lipids 2.2 in the liver and 4.5 in the heart, and for phospholipids, absent in the liver and 2.4 in the heart. The authors concluded that none of the examined organs accumulated erucic acid and that neutral fats incorporated more erucic acid than phospholipids. In another experiment, the same diet was fed to adult male rats for 45 days, followed by a rapeseed oil-free diet for 20 days in order to determine the elimination half-life. The half-life of erucic acid in the total lipids of various organs (lung, intestine, skin and carcass) and fat depots (perirenal, mesenterial and genital) ranged from 18 to 31 days and was about the same as for common fatty acids.

Carroll (1962) reported that the rat adrenal, which was not among the tissues studied by Wagner et al. (1958), accumulated large amounts of cholesteryl erucate when erucic acid was included in the diet. In support of this observation, Walker (1972a) reported that greatest deposition of erucic acid occurred in the adrenals and decreasing amounts were found in the plasma, heart, spleen, kidney, erythrocytes, testis and brain of male weanling Wistar rats fed a diet containing HEAR oil or a mixture of ethyl erucate and corn oil (5:1) for 18 weeks. Walker et al. (1972) further reported that female weanling Wistar rats maintained for 10 weeks on a diet containing a 5:1 mixture of ethyl erucate and corn oil extensively deposited erucic acid in the cholesteryl ester fraction of adrenals and ovaries, accounting for up to 30% of the total fatty acids. The authors concluded that this effect was related to the high activity of these organs for the synthesis of steroidal hormones.

In contrast to the rat studies, no preferential deposition of erucic acid in the tissue lipids from adrenals and ovaries were noted when 5-week-old swine were maintained for 6 weeks on a diet consisting of 59% corn and 25% soybean meal, and containing either 10% rapeseed oil or 10% corn oil (control). The greatest deposition of erucic acid, accounting for more than 7% of the total fatty acids, was found in plasma and adipose tissue lipids, whereas erucic acid comprised 3–5% of the total fatty acids in the spleen, adrenal, erythrocyte and heart. Only 1–3% erucic acid was found in the ovary, liver, kidney and testis (Walker, 1972b).

In all these studies, the amounts of erucic acid deposited in the various tissue lipids were quantified. However, because of the chain shortening of erucic acid during β-oxidation, exact figures for the initial distribution of erucic acid into different organs after absorption may not to be derived from the analysis of erucic acid alone. Even radiotracer studies may lead to erroneous results if the label can be removed during β-oxidation, such as with ^{14}C-erucic acid carrying the label near the carboxyl group.

The uptake of low concentrations of erucic acid, which did not perturb the normal plasma levels of fatty acids, into the liver and heart of male Sprague–Dawley rats was determined following infusion of 14-^{14}C-erucic acid (Murphy et al., 2008). A 2.3-fold higher uptake by the liver as compared with the heart was observed. This higher hepatic uptake was accounted for by a 4.2-fold higher incorporation of label into neutral lipids of the liver than those of the heart. In the liver, 56% of the label was found in cholesteryl esters, while in the heart 64% was found in triacylglycerols. Esterified neutral lipids of the heart contained 75% of the label as erucic acid and 10% as oleic acid. In contrast, only 25% were unchanged erucic acid, 10% were oleic acid and 50% were stearic acid in the liver. These data

indicate different metabolism of erucic acid in the liver (mainly conversion to stearic acid followed by export in neutral lipid) and heart (mainly incorporation into triacylglycerols).

Newborn Albino rats were kept on a diet containing 10% rapeseed oil (with 43% erucic acid) or blended canola oil (with 0.4% erucic acid). At the age of 4 months, the plasma, liver, adrenal gland, retina and brain were collected from both groups, and the fatty acids were determined in various lipid fractions. Samples from the retina were collected since rat retina is known to accumulate certain C22 polyunsaturated fatty acids, in particular docosahexaenoic acid (DHA), in the phospholipids of the rod outer segments (Fliesler and Anderson, 1983). Only in the rats receiving rapeseed oil, erucic acid was incorporated into lipids of the plasma (2.3%), liver (0.6%) and adrenal gland (17.6%) but not of the retina or brain, suggesting that erucic acid is not distributed into the latter two tissues (Wang et al., 1992).

There has been a long lived tenet according to which fatty acids do not readily penetrate the blood–brain barrier and consequently are not used as a source of energy by the brain, because they are bound to albumin and plasma lipoproteins. However, experimental studies have demonstrated that non-esterified fatty acids, readily liberated from albumin or hydrolysed from the glycerol backbone, are available for passing through the blood–brain barrier (Hamilton, 1998). According to a more recent hypothesis, fatty acids are not used for energy production in the brain because they have higher oxygen expenditure and cause more oxidative stress than glucose (Schönfeld and Reiser, 2013).

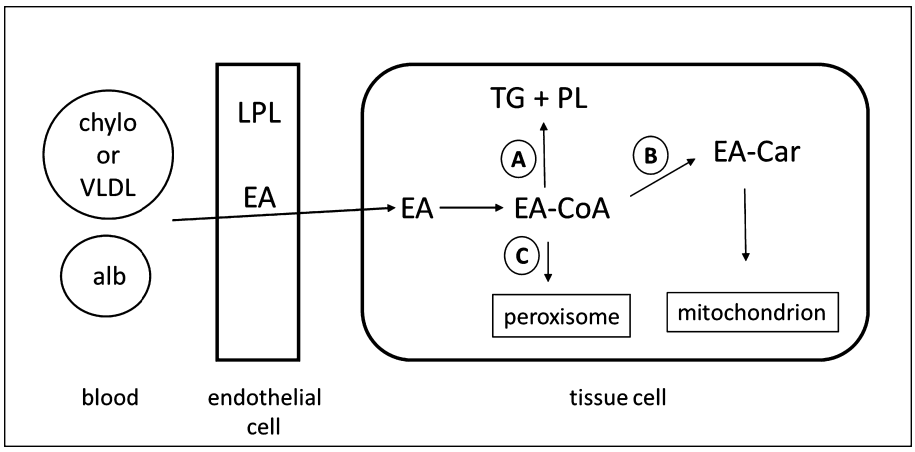
There are contradictory findings regarding the passage of erucic acid from the blood into the brain. Poulos et al. (1994) reported that increased levels of erucic acid were found in the fatty acids of the plasma and liver, but not of the *post-mortem* brain of adrenoleukodystrophy (ALD) patients treated with Lorenzo's oil.[35] Similar findings in ALD patients treated with Lorenzo's oil were reported by Rasmussen et al. (1994). Substantial amounts of erucic acid were present in the *post-mortem* adipose tissue and liver but not in the *post-mortem* brain. Likewise, feeding Wistar rats for 18 weeks with corn oil supplemented with ethyl erucate did not result in increased erucic acid concentrations in the brain (Walker, 1972a). In contrast, in a later study by Golovko and Murphy (2006), ^{14}C-erucic acid was used to probe the transfer of erucic acid from plasma into brain. After intravenous infusion of male Sprague–Dawley rats for 10 min, 0.01% of the plasma radioactivity was taken up by the brain and incorporated into triacylglycerols, phospholipids and cholesteryl esters. Moreover, about 60% of the radioactivity in the brain was attributed to eicosenoic and oleic acid. This study demonstrates that erucic acid crosses the blood–brain barrier to a small extent, is incorporated into specific lipid pools, and undergoes β-oxidation in the brain.

The fetal heart utilises glucose as its major energy source, and might therefore be protected from the cardiotoxic effects of erucic acid. Intrauterine mitochondrial fatty acid oxidation is not believed to play a major role. However, lethal fetal cardiac myopathy has been described, where impaired placental fatty acid oxidation has been proposed as an underlying cause (Rakheja et al., 2002; Oey et al., 2006).

3.3.1.3. Metabolism of erucic acid

As shown in Figure 4, free erucic acid in the blood is transported to the tissues as albumin complex, while erucic acid present in triacylglycerols of chylomicrons or very low-density lipoproteins (VLDL) is liberated by lipoprotein lipase located in the epithelial lining of the blood vessels (Feingold and Grunfeld, 2015). In tissue cells, the general fate of free erucic acid is activation to erucoyl-CoA, which can be used for the incorporation of erucic acid into triacylglycerols and phospholipids by esterification (route A) or for β-oxidation in mitochondria (route B) or for chain shortening in peroxisomes (route C). The conversion of erucoyl-CoA to erucoyl-carnitine is required for uptake into mitochondria. A forth and minor route (not depicted in Figure 4) is the chain elongation of erucic acid to nervonic acid (24:1 n-9). All four routes are mediated by enzymes. The metabolites resulting from chain shortening and chain elongation of erucic acid are depicted in Figure 5.

[35] Lorenzo's oil, a drug used for adrenoleukodystrophy (ALD), is comprised of 20% glycerol trierucate (a triacylglycerol with three erucic acid molecules) and 80% glycerol trioleate.



Chylo: chylomicrons; VLDL: very low-density lipoproteins; alb: albumin; LPL: lipoprotein lipase; TG: triacylglycerol; PL: phospholipid; Car: carnitine.

Figure 4: Cellular uptake and fate of erucic acid (EA)

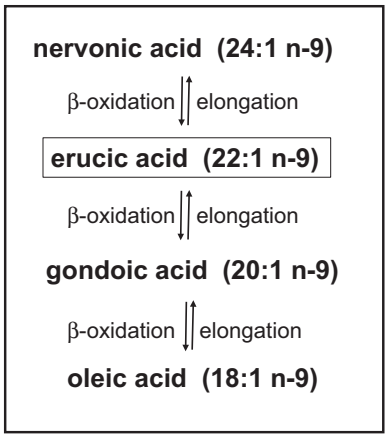


Figure 5: Products resulting from chain elongation and chain shortening of erucic acid

The numerous studies aiming at elucidating the metabolism of erucic acid in the heart and liver have been reviewed by Bremer and Norum (1982). Briefly, rats and other animal species have a limited capacity to β-oxidise erucic acid (and other fatty acids with more than 18 carbon atoms) in mitochondria due to a slow oxidation of erucoyl-CoA by the mitochondrial acyl-CoA dehydrogenase. Moreover, erucoyl-CoA has an inhibitory effect on the oxidation of the CoA esters of other fatty acids. As a consequence (which is discussed in more detail in Section 3.3.4.1), a diet containing high amounts of erucic acid causes an accumulation of triacylglycerols (lipidosis) in the heart and other tissues but not the liver, which is able to export erucic acid as VLDL to the blood plasma.

Because peroxisomes lack a short-chain acyl-CoA oxidase, complete degradation of erucoyl-CoA does not occur in peroxisomes. Only one or a few β-oxidation cycles take place, leading mainly to gondoic acid (20:1 n-9) and oleic acid (18:1 n-9) (Osmundsen et al., 1979). However, these products of peroxisomal degradation of erucic acid can be further β-oxidised in mitochondria.

In mitochondria, each acetyl-CoA molecule obtained by β-oxidation of erucic acid is dehydrogenated in the mitochondrial citrate cycle to two carbon dioxide molecules with formation of one flavin adenine dinucleotide ($FADH_2$) and three nicotinamide adenine dinucleotide (NADH) molecules, which enter the mitochondrial respiratory chain for adenosine triphosphate (ATP) production. The respiratory chain also utilises the $FADH_2$ and NADH generated during mitochondrial β-oxidation of erucic acid. Peroxisomes lack the citrate cycle and respiratory chain, and do not form $FADH_2$ but hydrogen peroxide (H_2O_2) during β-oxidation. The acetyl-CoA and NADH generated during chain shortening in peroxisomes are exported to the mitochondria for further utilisation.

It appears to be a general feature in all species that the mitochondrial oxidation rate of fatty acids decreases abruptly when the chain length exceeds 18 carbon atoms. For example, the oxidation rate of 22:1 acylcarnitines is 30–35% of that of 16:0 or 18:1 acylcarnitines in isolated heart mitochondria from pigs and oxen (Osmundsen and Bremer, 1978). However, there appear to be species differences in the ability of heart mitochondria to oxidise carnitine esters of fatty acids of varying chain length. Buddecke et al. (1976) observed that pig heart mitochondria oxidised erucic acid with ca. 3-fold higher rates than rat heart mitochondria. Moreover, Osmundsen and Bremer (1978) reported that 22:1 fatty acids inhibit the tricarboxylic acid cycle in heart mitochondria from the rat but not from the pig. Thus, overall respiratory depression was somewhat less severe in pigs than in rats.

The low capacity of heart mitochondria for β-oxidation of erucic acid observed in various animal species has also been demonstrated for humans (Clouet and Bézard, 1979). Mitochondria isolated from fragments of human heart appendages, removed during surgical intracardiac operations, were incubated with 14-^{14}C-erucic acid or 10-^{14}C-oleic acid. The radioactive products soluble in perchloric acid, that result from the β-oxidation, were formed in much lower amounts (about 6- to 10- fold) from erucic acid than from oleic acid. Activation of fatty acids, the initial step of β-oxidation, was also very much lower (about 5- to 7-fold) with erucic acid than with oleic acid (Clouet and Bézard, 1979). However, no information is available on cardiac lipidosis in persons ingesting erucic acid, e.g. patients receiving Lorenzo's oil.

The complexity of the metabolism of erucic acid and other fatty acids is increased by the fact that different organs may use the options of incorporation into lipids, chain degradation and chain elongation to various extents. This is illustrated by a study reported by Clouet et al. (1980) on the *in vivo* conversion of erucic acid into total lipids, mitochondria and microsomes from the liver, kidneys and heart of rats, 8 min after intravenous injection of albumin-bound 14-^{14}C-erucic acid. In the liver (containing 15% of the injected radioactivity), oleic acid (18:1 n-9) was the main fatty acid formed (26% of the ^{14}C recovered). In kidneys (0.53% of the injected radioactivity), the level of nervonic acid (24:1 n-9) was higher (20%) than that of oleic acid (14%). No appreciable transformation was encountered in the heart, which contained 0.53% of the injected radioactivity. In the liver, the microsomes showed higher converted radioactivity (45%) mainly as oleic acid (33%), much higher than in mitochondria (11%), whereas the amount of total ^{14}C fatty acid was a little higher in the latter fraction. In kidneys, the mitochondrial and microsomal fractions contained the same percentage of ^{14}C-oleic acid (15%), whereas nervonic acid was recovered in higher proportion, 29% in microsomes and 20% in mitochondria. In the heart, the mitochondrial and microsomal fractions contained the same low percentage of ^{14}C-oleic acid and nervonic acid (around 5%).

3.3.1.4. Excretion of erucic acid

Data on the excretion of erucic acid are extremely limited. Only older studies are available which have focussed on measuring the faecal excretion of erucic acid. As erucic acid can be metabolised by chain shortening and/or elongation (see Section 3.3.1.3), it would be necessary to use appropriately radiolabelled erucic acid to account for excreted metabolites.

Ziemlanski et al. (1973) administered a single dose of 0.8 mL of erucic acid ethyl ester (corresponding to 560 mg erucic acid) or 1.6 mL of rapeseed oil (erucic acid content not given) either alone or mixed with soybean oil. The time course of faecal excretion of erucic acid was similar after each dosing regimen, with the major proportion being excreted within 48 h and completion reached after 4–5 days. About 30% of the dosed erucic acid was accounted for in the 5-day faeces when erucic acid ethyl ester or rapeseed oil had been given alone, and about 20% when given together with soybean oil.

3.3.1.5. Summary regarding the absorption, distribution, metabolism and excretion of erucic acid

Erucic acid is present in food and feed, predominantly as component of triacylglycerols. It is well absorbed from the gastrointestinal tract to an extent varying between 60% and 100%, depending on the species. Humans exhibit virtually complete absorption. Like other fatty acids, erucic acid is distributed to the different organs where it is metabolised by the following major pathways: (1) incorporation into lipids (mostly triacylglycerols), (2) complete β-oxidation in mitochondria, (3) partial β-oxidation (chain shortening) in peroxisomes, and (4) chain elongation. In contrast to fatty acids with 18 or less carbon atoms, erucic and other docosenoic acids are poorly metabolised by β-oxidation in mitochondria and depend on chain shortening in peroxisomes, in particular in rats but also in pigs.

3.3.1.6. Transfer into food of animal origin

In many of the studies identified, the levels of erucic acid in food of animal origin were only reported as percentage of total fatty acids, and therefore, transfer from feed to final product could not be calculated except in milk. The CONTAM Panel noted that several of the studies were reported 40 years or more ago, and therefore, the applicability to current animal genotypes is uncertain.

Meat

Diets supplemented with high (23.7%) or low (2.4%) erucic acid oils were fed to broiler chickens for 4 weeks (Vogtmann and Clandinin, 1974). There were significant differences ($p < 0.001$) in the erucic acid contents of different tissues from the broiler chickens; in breast tissue, for example, erucic acid content were 6.8% and 0.7% of fatty acids in broiler chickens fed the high and low erucic acid diets, respectively. Feed intakes were not given, and therefore, transfer rates cannot be calculated.

In a subsequent study broiler chickens were fed diets supplemented with oil (15% inclusion in the diet) from different varieties of LEAR oil, for 8 weeks. Erucic acid contents of the oils ranged from < 0.1% to 3.5% of total fatty acids. Erucic acid contents ranged from 'undetected' to 1.2% of total fatty acids in spleen from broiler chickens fed a refined oil. The authors concluded that 'only small amounts of erucic acid were found in the tissues' (Vogtmann et al., 1974).

Farnworth et al. (1994) examined the effect on the body composition of piglets as a result of feeding artificial milk containing 0%, 2%, 7%, 12% or 20% erucic acid. Digestibility of erucic acid was high (> 98%), and the erucic acid content of the backfat[36] reflected the concentrations in the diet. In the pigs receiving the highest erucic acid diet, the level of erucic acid in backfat was approximately 18% of total fatty acids; when the erucic acid content was reduced to 2%, this level was only 0.6%. Erucic acid was also deposited in adipose tissue.

Böhme et al. (2005) undertook a study in which crambe press cake was fed to growing and fattening pigs (24–120 kg bw). Erucic acid contents in the diets ranged from 0.2 to 7.1 g/kg dry matter (DM), and the total intake of erucic acid over the growing and fattening periods ranged from 47 to 1,912 g. At the three lowest levels of intake (47, 80 and 173 g erucic acid over the experimental period), there were no significant differences in the erucic acid contents of backfat or intramuscular fat. At the two highest intakes of erucic acid there was a significant ($p < 0.05$) dose-related increase in the erucic acid content (as a % total fatty acids) from < 0.1% (control treatment) to 1.5% in backfat and 0.7% in intramuscular fat.

Milk

Böhme et al. (2005) examined the transfer of erucic acid from feed to milk. Mid-lactation dairy cows were fed diets containing 0%, 15% or 30% crambe press cake for 8 weeks. Erucic acid contents were 0.1%, 1.3% and 2.1% of total fatty acids in milk ($p < 0.05$). There was a direct and positive correlation between erucic acid output in milk and erucic acid intake, and from the data provided for erucic acid intake and milk fat production the CONTAM Panel calculated transfer rates of 100%, 26% and 16%, respectively.

More recently, Hristov et al. (2011) fed lactating dairy cows diets containing conventional, solvent-extracted canola meal or high erucic acid (erucic acid 42% of total fatty acids), low-glucosinolate rapeseed meal. Rumen studies indicated that erucic acid is partially hydrogenated or isomerised in the rumen, and that the resulting biohydrogenation intermediates and end-product fatty acids are readily absorbed and utilised in the mammary gland for milk fat synthesis. As a result, replacing conventional rapeseed meal with HEAR meal resulted in a significant increase in erucic acid from 0.07 to 2.33 g/kg total fatty acid in the milk ($p < 0.001$).

In both these reports, erucic acid was expressed as % of total fatty acids. In order to convert from % in total fatty acids to % in milk fat, it is necessary to account for the glycerol present in milk, and the accepted conversion factor for milk fat is 0.945 (Paul and Southgate, 1978). Adopting this approach, the erucic acid content of milk ranged from 0.02 to 0.65 g/kg in Hristov et al. (2011), and from 0.04 to 0.8 g/kg milk in Böhme et al. (2005).

Eggs

A number of studies have examined the transfer of erucic acid from feed to the fatty acids in egg yolks. Lall and Slinger (1973) fed laying hens diets supplemented with 10% or 20% of HEAR and LEAR

[36] The panniculus adiposus along the back of a pig.

oils (containing approximately 32% and 1.2% erucic acid, respectively). While the total lipid content of the yolk was not influenced by the type or level of dietary fat, the addition of the different rapeseed oils altered the fatty acid composition of egg yolk. The average erucic acid content of yolk fat of hens fed 10% and 20% of the HEAR oil was 0.6%, but was 0.2% in yolk fat of eggs from laying hens fed the LEAR oil.

Vogtmann et al. (1974) examined the effects of including 5% or 15% of LEAR and HEAR oils on the total lipid and fatty acid contents of egg yolks. The erucic acid content of egg yolk fatty acids was 0.2% in eggs from laying hens fed 15% of the HEAR oil, but was < 0.1% in the fatty acid of egg yolks of laying hens fed the LEAR oil. Based on other changes in the fatty acid contents of the egg yolks, the authors concluded that in the laying hens a partial degradation of erucic acid occurs.

Honey

The CONTAM Panel considered the transfer of erucic acid from pollen into honey. Evans et al. (1988) reported the fatty acid composition of pollen and seeds from three cultivars of *B. napus*, including one cultivar high in erucic acid. Only traces of erucic acid (< 2.0% of the fatty acids) were found in the pollen. Usually, the total lipid content of pollen is less than 10% (Nicolson, 2011), however, also higher levels have been reported (e.g. 23.8% of dry weight, Evans et al., 1988). In addition, it should be noted that the pollen content in honey is low. Puusepp and Koff (2014) reported an average concentration of 10,000 grains/g honey and considering that 2,000 pollen grains weigh 1 mg (Porter, 1981), the pollen content in honey is around 0.5%. Given the low amounts of pollen in honey, the low lipid content in pollen and the low fraction of erucic acid in the lipid fraction of the pollen, the CONTAM Panel concluded that the transfer of erucic acid from pollen into honey is negligible.

Fish

Torstensen et al. (2004) fed Atlantic salmon diets in which fish oil was replaced by 25%, 50%, 75% or 100% rapeseed oil, giving erucic acid (22:1 n-9) contents ranging from 2.0% (100% fish oil) to 0.5% (100% rapeseed oil). Levels of erucic acid in both red and white muscle and livers declined with declining levels in the diet.

Summary

There is evidence that erucic acid present in feed is transferred to products of animal origin and a dose-related increase in erucic acid in food of animal origin has been shown. With the exception of the study reported by Böhme et al. (2005), it has not been possible to estimate transfer rates from feed to food of animal origin. In ruminants, erucic acid is also partially hydrogenated or isomerised in the rumen.

3.3.2. Toxicity in experimental animals relevant for human risk assessment

3.3.2.1. Acute toxicity (single dose)

Groups of five male and female Wistar rats received a single erucic acid dose of 5 g/kg bw as a 25% suspension in water by gastric intubation (Henkel KGaA, 1981a). The animals exhibited a rough fur and reduced motor activity shortly after dosing. These effects were slight and disappeared within 24 h. All animals gained body weight and survived an observation period of 14 days without symptoms of toxicity. The histological examination of internal organs did not reveal any abnormalities except an acute inflammation of the intestine. The authors concluded that the oral LD_{50} of erucic acid for male and female Wistar rats is > 5 g/kg bw. Although the study was conducted according to OECD guideline 401, there was no control group and the organs for histological examination were not specified.

3.3.2.2. Repeated dose toxicity

The animal species most widely used for studying the toxicity of erucic acid is the rat. In most studies, rapeseed or mustard oil with a high or low content of erucic acid, present in the fraction of triacylglycerols, has been used rather than glycerol trierucate or erucic acid *per se*. It should be noted that several studies used rapeseed oils high in erucic acid while rapeseed oil available on the European market for dietary use contains usually extremely low levels (less than 0.5%) of erucic acid. Results reported in Sections 3.3.2.2, 3.3.2.4, 3.3.2.5 and 3.3.5 for rapeseed oil are therefore not representative for rapeseed oils containing low levels of erucic acid that are available on the European market for dietary use.

A. Studies including only low erucic acid rapeseed oils in experimental animals

As defined by the Codex Alimentarius Commission LEAR oils contain less than 5% erucic acid (Codex Alimentarius Commission, 1979). Their toxicity has been extensively reviewed by Kramer and Sauer (1983b). The LEAR oil presents a particular fatty acid composition: it is rich in oleic acid (18:1 n-9), similarly to peanut and olive oil, and presents also a relatively high content of linolenic acid (18:3 n-3), similarly to soybean oil (Kramer et al., 1979a). Typical ranges of variation for both fatty acids, considering broad variation for cultivar and environmental conditions, are between 54% and 71% for oleic acid and between 3% and 14% for linolenic acid (Seberry et al., 2006, 2014). The digestibility of LEAR oil in rats is very high, approaching 100%.

A large body of evidence indicates that LEAR oils do not cause myocardial lipidosis in rats; however, a high incidence of myocarditis in male rats is reported on prolonged feeding of this rapeseed oil. The extensive literature review by Kramer and Sauer (1983b) allows concluding that LEAR oils consistently produce a higher incidence of lesions in rats than other oils. The severity of the lesions (number of lesions per heart) is also, in general, significantly higher in rats fed LEAR oils than other oils. Analysis of published data (Kramer and Sauer, 1983b) in rats indicate that, of the fatty acids in vegetable oils, 18:1, 18:3, 20:1 and 22:1 promote the occurrence of myocardial lesions whereas 16:0, 18:0 and 18:2 have a protective effect. Therefore, the fatty acid composition (particularly the presence of oleic acid and linolenic acid) of LEAR oils may account for the incidence of myocarditis induced in rats by this vegetable oil. Pigs, dogs, mice and non-human primates do not respond to dietary LEAR oils like the rat, showing heart lesions that are in general not related to fat and likely of different aetiology.

Effects on the liver (e.g. morphological alterations) and platelet number have been reported in piglets (Cullen et al., 1996; Innis and Dyer, 1999), but as in the case of cardiotoxicity these effects cannot be ascribed to erucic acid because of the fatty acid composition of LEAR oils.

Therefore, the CONTAM Panel concluded that studies using only LEAR oils cannot be used for the risk assessment of erucic acid.

B. Studies including oils high in erucic acid (> 5%)

Rat

The studies in rats with only high erucic oils or with both high and low erucic acid oils are described in Appendix G, Table G.1 (including the doses). The text below summarises the toxic effects observed in these studies.

It is to be noted that in these experiments, rats were fed diets containing a high amount of fat (20–30%), which already may have an effect on the rats compared to rats fed standard (low-fat) diets: e.g. reduced food consumption, increase in body weight, modifications of levels of some nutriments, induction of signs of myocardial lipidosis (Abdellatif and Vles, 1973a; Engfeldt and Gustafsson, 1975; Ziemlanski et al., 1975; Kramer et al., 1979b; Kramer et al., 1992; Vaskonen et al., 1996).

Cardiac lipidosis

The heart is the principal target organ of toxic effects following short or long-term exposure of rats to diets containing erucic acid. Myocardial lipidosis is produced in rats fed diets containing rapeseed oil, mustard oil or methyl erucate (e.g. Beare-Rogers et al., 1971; Rocquelin et al., 1975; Kramer et al., 1992). Lipidosis appears early after feeding (e.g. Abdellatif and Vles, 1970a,b, 1973a; Houtsmuller et al., 1970; Beare-Rogers et al., 1971; Ziemlanski et al., 1975); lipid droplets may be detected in myocardial cells by electron microscopy in rats fed HEAR oil as early as 3 h after feeding (Ziemlanski et al., 1973). Myocardial lipidosis reaches a peak 3–7 days after onset of feeding and regresses thereafter. The severity and duration of this alteration are directly related to the erucic acid content of the diet and the duration of exposure (Corner, 1983; Kramer et al., 1988). Myocardial lipidosis regresses following long-term administration of high doses of erucic acid, but still remains above that found in control animals at the end of the exposure period (e.g. Beare-Rogers et al., 1972b; Abdellatif and Vles, 1973a). In addition, Kramer et al. (1988) have shown that lipidosis induced in rats after 1 week exposure to a high-fat/high erucic acid diet was reversible to nearly control levels within 1 week when changing to a low-fat diet without erucic acid. One of the possible mechanisms for the myocardial triglyceride accumulation and its regression is an increased ability of the heart to oxidise erucic acid to shorter chain fatty acids (see Section 3.3.4) (Kako and Vasdev, 1979; Norseth et al., 1979). Electron microscopic examinations showed that lipid droplets were closely associated with mitochondria. Houtsmuller et al. (1970) have described the impaired oxidative capacity of the heart

mitochondria after exposure of rats for 6 weeks to HEAR oil. Mitochondrial damage (megamitochondria, mitochondrial proliferation, increase in the average volume, distortion of shape, degeneration of crista), and disorganisation of myofibrils have been reported after exposure of male rats to rapeseed oil for several months (Bhatnagar and Yamashiro, 1979; Yamashiro and Clandinin, 1980). Charlton et al. (1975) reported that cardiac lipidosis in rats fed a variety of rapeseed oil was more severe in the ventricular walls and interventricular septum than in the atrial walls. Myocardial lipidosis is reported to reduce the contractile force of the heart muscle. ten Hoor et al. (1973) showed that myocardial function of rats fed HEAR oil for a short term (i.e. 3 days) is reduced. They suggested that the decreased contractile force of the heart muscle may be related to an impaired mitochondrial function that decreased capacity to oxidise substrate and a decreased rate of ATP synthesis. Other authors reported no functional changes (Kako and Vasdev, 1979; Kako et al., 1980). Heart lipids of rats exposed to HEAR oil were rich in erucic acid. Specific accumulation of triacylglycerols as well as an increase in cardiac free fatty acids was observed in the heart of rats fed HEAR oils (Kramer et al., 1992). Cardiac triacylglycerols are readily mobilised for energy production or serve as phospholipids precursors (Kramer and Sauer, 1983a). Cardiac lipidosis was not observed, or only traces, in rats fed lard, sunflower, corn or peanut oil. The presence of intracellular fat droplets was also observed in skeletal muscles of rats fed HEAR oil. Pale heart and muscles were described in rats fed HEAR oil (Corner, 1983).

It has been shown that myocardial lipidosis of rats fed HEAR oil, partially hydrogenated HEAR oil, herring oil or partially hydrogenated herring oil is correlated with the amount of docosenoic acids[37] (in particular erucic acid, cetoleic acid and brassidic acid) in cardiac lipids (Beare-Rogers et al., 1972a). Studies have also shown that greater lipid deposition occurs in weanling rats fed rapeseed oil than in similarly treated older rats.

The CONTAM Panel considered that lipidosis is a relevant effect for the risk assessment of erucic acid.

Degenerative lesions in the heart

Ziemlanski et al. (1975) have shown that exercise contributes to the utilisation of great surplus of myocardial erucic acid. Myocardial fatty degeneration was less intense and the erucic acid percentage of total fatty acids in the heart was lower in trained rats fed a diet containing very high levels of erucic acid than in untrained rats. Training was also found to reduce the growth rate of the animals.

After 2–4 weeks feeding with diets containing rapeseed oil, degenerative lesions of cardiac muscular fibres appear in rats, followed by myocardial necrosis (death of myocardial cells), removal of the necrotic cellular debris by macrophages and repair of the lost muscle by a fibrous connective tissue scar (fibrosis, observed mainly from 16 weeks feeding) (Ziemlanski et al., 1975). Myocardial necrosis may occur in rats subjected to many different nutritional and metabolic conditions and is influenced by many factors (age, sex, strain, source); the male being more severely affected (Kramer et al., 1988) and at an earlier age. Cardiac injury might be due to an interaction of male sex hormones with specific dietary fat components. Kramer et al. (1988) have shown that growth rate is also involved in the aetiology of myocardial necrosis in rats; small male showing significantly less myocardial necrosis than heavier rats. Myocardial lesions have been observed in the hearts of rats fed rat chow, control oils such as corn, peanut, olive, soybean or various marine oils. Therefore, myocardial necrosis is considered to be a spontaneous idiopathic lesion in the male rat. Experiments done by Innis and Clandinin (1980) have shown that fundamental physiological differences in the profile of fatty acids (saturated and essential fatty acids) reaching the heart occur between young growing male rats fed for 1 week and those fed for 4 weeks and between male and female rats fed identical fats for the same length of time. In females greater movement of fatty acid through hepatic and extrahepatic tissue may result in modification of plasma fatty acids. These results suggest that physiological differences in whole body fat metabolism unrelated to plasma fatty acids determine strain differences in timing and severity of rapeseed oil-induced cardiac pathology. Charlton et al. (1975) reported that cardiac necrosis and fibrosis in rats fed a variety of rapeseed oils was more severe in the ventricular walls and interventricular septum than in the atrial walls. Acute and chronic lesions are sometimes present in the same heart, suggesting that focal necrosis does not occur at one time but is continuous or recurrent during at least part of the feeding trials. In some experiments, higher incidence and severity of myocardial necrosis have been observed in rats fed HEAR oil compared to control oils or

[37] Docosenoic acid is the group name for all fatty acids with 22 carbon atoms and one double bound regardless of the position of the double bound and *cis* or *trans* configuration; e.g. erucic acid, cetoleic acid and brassidic acid.

LEAR oil (Hung et al., 1977). Several hypotheses have been proposed to explain the aetiology of the focal myocardial necrosis and fibrosis seen in male rats following feeding with various rapeseed or herring oils. Some authors considered that erucic acid alone was responsible for the production of the focal necrosis (Abdellatif and Vles, 1973a; Engfeldt and Brunius, 1975b; Astorg and Cluzan, 1976). However, the results of several studies suggested that the increased incidence of myocardial necrosis was the result of a fatty acid imbalance (relative abundance of saturated and/or unsaturated acids) for the growing male albino rat (Kramer et al., 1973, 1975; Hulan et al., 1977). Common C16 and C18 fatty acids found in most vegetable oils appear to be involved in cardiac necrosis of the rat (Kramer et al., 1975). According to Kramer et al. (1988), the development of myocardial necrosis appears to be caused by a combination of factors such as an alteration of cardiac phospholipids and/or their fatty acid composition (saturated fatty acids, C22 n-3 Polyunsaturated fatty acids (PUFAs) and long chain monoenes of 20:1, 22:1 and 24:1). Several studies have shown that there is no apparent relationship between the number of lesions per heart and the amount (accumulation) of cardiac triacylglycerols and free fatty acids or the concentration of erucic acid in the cardiac triacylglycerols or free fatty acids in the heart of Sprague–Dawley rats (Kramer and Hulan, 1978; Kramer et al., 1979b).

The CONTAM Panel concluded that, from these studies with oils, myocardial necrosis cannot be specifically linked to erucic acid, and therefore, myocardial necrosis is not a suitable endpoint for risk assessment.

Other cardiac endpoints

Lisciani et al. (1989) have shown that the exposure of Wistar male rats for 10 days to erucic acid (11.4 g/kg bw per day) results in an earlier occurrence of ventricular fibrillation, pulmonary oedema (increased incidence) and cardiac arrest compared to the control group after being injected with adrenaline until cardiac arrest. The development of pulmonary oedema was the result of the inadequacy of cardiac output to counterbalance the increase in vascular resistance induced by adrenaline. A diet rich in erucic acid was more arrhythmogenic and the heart was less efficient. However, no effect on the electrocardiogram (ECG) was observed after feeding rats with HEAR oil for 8 weeks (Berglund, 1975) or with HEAR oil or erucic acid for 26 weeks (de Wildt and Speijers, 1984). So, in spite of gross morphological changes, no disturbances seemed to occur within the conductance system of the affected heart. Although focal myocardial fibrotic lesions was induced by HEAR oil after 24–26 weeks feeding, no changes were noted with respect to the intrinsic myocardial contractibility *in vitro* and *in vivo*. After inotropic intervention, only the HEAR oil fed rats showed less contractile reserve capacity. This was not observed in the erucic acid-treated rats which do not show epicardiac fibrotic lesions. The authors concluded that rapeseed oil and not erucic acid is responsible for loss of contractile reserve capacity without changes in the myocardial conductance system and that erucic acid might interfere with the peripheral vascular system, reducing vascular reactivity. They showed also that erucic acid alone appears to be responsible for an impaired vasoconstrictor response towards noradrenaline. It appears also that a fat rich diet might result in reduced myocardial function during a state of energy demand coupled with a blood pressure decrease (de Wildt and Speijers, 1984).

When the hearts taken from rats fed for 10 days with a normal or erucic acid diet (12 g/kg bw per day) were perfused aerobically with an isovolumic preparation, no difference was observed in mechanical activity (heart weight, rate at which the isolated heart beat or the coronary perfusion pressure) between the two groups of rats. However, when pressure-volume curves were determined in the paced hearts, the pressure developed by hearts from erucic acid fed rats was reduced, indicating that erucic acid causes systolic as well as diastolic dysfunction (Pasini et al., 1992a,b).

Stewart et al. (1993) reported that the fat accumulation that occurs in isolated perfused hearts of rats fed diets rich in high erucic acid rapeseed oil does not interfere with the cardiac high energy phosphate metabolism or contractile function. However due to the limitations of the study (e.g. small number of animals, rate pressure product has been shown not to correlate with cardiac workload) by Aksentijevic et al. (2016), it was not further considered.

Non-cardiac effects

Other effects observed in rats exposed to HEAR oil are listed below:

- decreased feed intake, weight gain from about 6–8 g/kg bw per day (4–16 weeks feeding) (e.g. Craig et al., 1963; Beare-Rogers et al., 1971; Ziemlanski et al., 1975; Hung et al., 1977; Chun et al., 1988);

- changes in the liver (increased weight, fat accumulation, change in lipid composition, accumulation of erucic acid, hepatocytes vacuolation, mononuclear cell aggregation) from about 4 g/kg bw per day (Ziemlanski et al., 1972a,b, 1975; Kramer et al., 1979b);
- changes in the kidneys (increased weight, degenerative changes, tubular dilatation, vacuolation of tubular epithelium, casts, scar formation) from about 6 g/kg bw per day (Abdellatif and Vles, 1970b, 1971b, 1973a);
- changes in skeletal muscles (fatty infiltration) from about 13 g/kg bw per day (Abdellatif and Vles, 1970b, 1973a);
- changes in adrenal (lipidosis, enlargement of cortical cells, modification of activities) from about 4 to 6 g/kg bw per day (Abdellatif and Vles, 1971b, 1973a; Ziemlanski and Budzynska-Topolowska, 1978b);
- increased testis weight at about 4 g/kg bw per day (Ziemlanski and Budzynska-Topolowska, 1978b).

Studies relevant for hazard characterisation

Due to the high number of studies available (see Appendix G, Table G.1), the CONTAM Panel defined four criteria for the identification of studies that are suitable for hazard characterisation, namely:

- testing of several doses with a wide dose-range;
- erucic acid being reported as the main source of variation in fatty acid composition of the diet;
- possible identification of a no observed adverse effect level (NOAEL)/lowest observed adverse effect level (LOAEL);
- observation of myocardial lipidosis as relevant toxic effect.

From the studies described in Appendix G, Table G.1, the CONTAM Panel identified six studies that met these criteria. These studies are summarised in Table 11 and are described in more detail below.

In a study by Kramer et al. (1992), young male Sprague–Dawley rats (10/group) were fed for 1 week diets containing 20% by weight fat/oil mixtures with different levels of erucic acid. The test oil mixtures were prepared by mixing canola oil, HEAR oil and cocoa butter to give oils with low (2.4% or 2.9%) and medium (8.7% or 10.1%) erucic acid content, each with a low (8%) or a high content (35%) of total saturated fatty acids. In addition, HEAR oil (42.9% erucic acid; high erucic acid group) and corn oil (control group) were tested. Based on the information available, the CONTAM Panel calculated the following doses of erucic acid: 0, 0.6–0.7, 2.1–2.4 and 10.3 g/kg bw per day using the default factor of 0.12 for a subacute study in rats (EFSA Scientific Committee, 2012). There were no significant differences in weight gain of rats fed the four oil mixtures and corn oil; however, rats fed a diet with HEAR oil gained significantly less weight. The heart weights were similar for all groups. Rats fed corn oil showed traces of myocardial lipidosis by histological staining (incidence: 6/10, severity 3 $\pm$ 1.2). A significant increase in severity of myocardial lipidosis as shown histologically and by an accumulation of erucic acid in heart lipids was observed at about 2.1 g/kg bw per day. Incidences of lipidosis were 8/10 & 10/10 in the low erucic acid groups, 10/10 & 10/10 in the medium erucic acid groups and 10/10 in the high erucic acid group. The severity (area %) was: 14 $\pm$ 5.6 & 13 $\pm$ 4.8, 56 $\pm$ 9.0 & 44 $\pm$ 5.6 and 100%, respectively. The areas most affected were the right and left ventricles near the base of the heart. Extensive myocardial lipidosis was observed throughout the whole heart in rats fed diets with HEAR oil. No increase in cardiac triacylglycerol was noted except in the rats fed the highest dose. No changes in myocardial lipid content was shown histologically by the content of cardiac triacylglycerol or the erucic acid content of triacylglycerol at 0.6–0.7 or 2.1–2.4 g/kg bw per day. A better correlation was noted between the histological staining method and the erucic acid content in cardiac triacylglycerol than with the total cardiac triacylglycerol content. The erucic acid content was the highest in cardiac triacylglycerol and free fatty acids. An increase in saturated fatty acids in the diet, did not change the incidence and/or severity of myocardial lipidosis at 0.6–0.7 or 2.1–2.4 g/kg bw per day. The CONTAM Panel identified from this study a NOAEL of 0.7 g/kg bw per day based on the increased severity of myocardial lipidosis at 2.1 g/kg bw per day (Kramer et al., 1992).

Beare-Rogers et al. (1971) reported two experiments of which one was identified by the CONTAM Panel based on the criteria described above. Varying levels (0%, 2.5%, 5%, 10%, 15% or 20% by weight) of rapeseed oil (containing 29.7% erucic acid) were fed for 1 week to weanling male rats (15/group; strain not reported) to produce maximal fat deposition. Other rats were fed diets

containing 10% or 20% canbra oil[38] (containing 2.9% erucic acid). Based on the information provided by the authors, the CONTAM Panel calculated doses of 0, 0.3, 0.7, 0.9, 1.8, 3.6, 5.3 or 7.1 g/kg bw per day, respectively when applying a default factor of 0.12 (EFSA Scientific Committee, 2012). Deposition of total fatty acid and erucic acid in the heart was absent at 0.3, 0.7, 0.9 and 1.8 g/kg bw per day but increased greatly in rats exposed to 3.6 g/kg bw per day or more. Accumulation of fatty acids occurred mainly as triacylglycerols. The two highest erucic acid doses were associated with deposition of fat globules within myocardial cells and between the myofibrils. Other changes were interstitial oedema, myocytolytic changes in the cardiac muscle cells and some focal areas of necrosis. The CONTAM Panel identified a NOAEL of 1.8 g/kg bw per day based on deposition of total fatty acid and erucic acid in the heart. The NOAEL for myocardial lipidosis was 3.6 g/kg bw per day.

In a study by Kramer et al. (1988), male Sprague–Dawley rats (3 weeks of age) were fed a low-fat diet (5% by weight corn oil and 45% starch) for 14 weeks followed by high-fat diets (20% by weight rapeseed oil and 30% starch) for 1 week containing graded levels of erucic acid: 1%, 10%, 20%, 30%, 40% and 50%. The CONTAM Panel calculated erucic acid doses in the diet of 0, 0.1, 1.6, 4.1, 5.2, 7.0 and 8.8 g/kg bw per day, respectively, when applying a default factor of 0.12 (EFSA Scientific Committee, 2012). Some rats within each group were returned to the low-fat diet for 1 week after the test period. For comparison, one group of 3-week-old male rats were fed the high-fat 50% erucic acid diet for 15 weeks. Growth depression and reduced feed intake was noted in rats fed diets rich in erucic acid. However, the results were confounded by an increase in body weight resulting from the switch from a diet containing 5% fat to one containing 20% fat. The growth depression effect of erucic acid was removed on return to a diet without erucic acid. Within 1 week of feeding rapeseed oil diets, myocardial lipidosis was induced in adult rats. A dose-related increase in severity was observed (relative lipidosis grading: no fat stain, very slight, slight, moderate, moderate and marked). This increase was reversible after return to low-fat diet without erucic acid (nearly control level). The switch also involved a reduction in the level of fat in the diet from 20% to 5% by weight, which might have expedited the regression of myocardial fat infiltration. Continuous feeding of the same HEAR oil diet reduced the severity of myocardial lipidosis significantly (moderate) compared to rats fed this diet for only 1 week. The relatively high residual lipidosis in male rats fed HEAR oil for 15 weeks may be explained by the high dietary concentration of erucic acid. A dose-related increase in the incidence of myocardial necrosis (not reported in control, 7/30, 9/30, 17/30, 15/30, 9/30 and 16/30, respectively) but not in severity was seen. The response to dietary erucic acid, however, was not linear. Long-term feeding of HEAR oil resulted in a much higher incidence and severity of myocardial necrosis (incidence: 20/20; severity: 18 rats with more than 6 lesions/heart) than the 1-week exposure. Continued feeding of a diet rich in erucic acid may have increased the rat's capacity to metabolise erucic acid as evidence by a lower level of erucic acid and higher levels of 20:1 n-9 and 18:1 n-9 compared with male rats fed the same diet for only 1 week. Based on their observations, the authors suggested that the development of myocardial necrosis appears to be caused by a combination of factors such as an alteration of cardiac phospholipids and/or their fatty acid composition (saturates, C22 n-3 PUFAs and long chain monoenes of 20:1, 22:1 and 24:1). The CONTAM Panel identified a NOAEL of 1.6 g/kg bw per day based on increased severity of myocardial lipidosis at 4.1 g/kg bw per day.

Abdellatif and Vles (1970a) performed a dose–response study in which Wistar male rats (3-week-old; 12/group) were given 0%, 5%, 10%, 15%, 20%, 25% or 30% by weight rapeseed oil (46% erucic acid) for 2 weeks. The CONTAM Panel calculated erucic acid doses in the diet of 0, 2.6, 5.5, 8.8, 12.6, 17.0, 22.1 g/kg bw per day, respectively, when applying a default factor of 0.12 (EFSA Scientific Committee, 2012). Growth rate slightly increased as erucic acid dose raised from 2.6 to 5.5 g/kg bw per day but decreased with further increases in rapeseed oil. An erucic acid dose of 5.5 g/kg bw per day was found as the minimum level to elicit fatty accumulation in the heart and in the skeletal muscles, which increase in severity with increasing doses. Hypertrophy of the adrenal cortical cells was observed at doses from 12.6 g/kg bw per day. The CONTAM Panel identified a NOAEL of 2.6 g/kg bw per day based on myocardial lipidosis.

In a study by Beare-Rogers et al. (1972b) male weanling COBS® rats (10–12/group) were fed diets containing 20% by weight fat with 0%, 2.5%, 5%, 10%, 15% or 20% rapeseed oil (38.1% erucic acid), corresponding to erucic acid doses of 0, 0.9, 1.7, 3.4, 5.1 and 6.9 g/kg bw per day for 1–16 weeks. Doses were calculated using a default factor of 0.09 (EFSA Scientific Committee, 2012). Other rats received 0–15% by weight partially hydrogenated rapeseed oil (35.2% 22:1) or 0–15% by weight partially hydrogenated herring oil (31.3% 22:1). Cardiac lipid (fatty acids) accumulation was

[38] Name previously used to refer to LEAR oil in Canada; currently canola is used.

noted in rats fed rapeseed oil and reached a peak at 1 week and decreased thereafter. The incidence of hearts positive for fat stain were 0/10, 0/10, 0/10, 5/10, 9/10 and 10/10 in the groups receiving doses of 0, 0.9, 1.7, 3.4, 5.1 and 6.9 g/kg bw per day, respectively. The accumulation of fatty acids appeared to be related to the concentration of docosenoic acid (22:1). The triacylglycerol fraction accounted for most of the deposited fat and contained a high concentration of docosenoic acid. After 16 weeks, degenerative lesions (necrosis and fibrosis) were observed in rats receiving rapeseed oil (0/12, 0/12, 3/12, 3/12, 6/12 and 9/11, respectively). Similar incidences of lesions were observed in rats fed with partially hydrogenated rapeseed oil or partially hydrogenated herring oil. The CONTAM Panel identified from this study a NOAEL of 0.9 g/kg bw per day based on degenerative lesions. The NOAEL for myocardial lipidosis was 1.7 g/kg bw per day.

Abdellatif and Vles (1973a) fed Sprague–Dawley rats (3-week-old; 8/group/sacrifice time) semisynthetic diets containing 20% by weight fat with different levels of rapeseed oil (0%, 2.5%, 5%, 7.5%, 12.5% or 15%). The diets were made isocaloric in fat by addition of sunflower seed oil. Based on the information available, the CONTAM Panel calculated the following doses of erucic acid: 0, 1.0, 2.0, 3.0, 4.0, 5.0 and 5.9 g/kg bw per day using the default factor of 0.09 (EFSA Scientific Committee, 2012). The rats were killed after 3 and 6 days and 32 weeks. The growth rate was lower in rats fed 5.9 g/kg bw per day. Significant differences in the absolute and relative weights of various organs where only observed when the erucic acid dose was $\geq$ 3.0 g/kg bw per day (spleen, kidneys from 3.0 g/kg bw per day and thyroid, heart, testis and liver at the highest dose). All animals fed rapeseed oil showed lipidosis of the heart, skeletal muscle, diaphragm and adrenals after 3 or 6 days and decreased thereafter. The lipidosis became more severe as the level of rapeseed oil increased. After 32 weeks, diet-related changes were observed in the kidneys (slight tubular dilatation and increased debris in the lumina, especially in rats fed 5.9 g/kg bw per day), adrenals (enlargement of the cortical cells from 2.0 g/kg bw per day) and the heart. The cardiac changes consisted of minimal lipidosis, foci of myocytolysis showing mononuclear cell proliferations, thickening of the reticular sheath around individual muscle fibres, increase in the interstitial connective tissue elements and aggregates of Anitskow cells. Minimal degrees of these changes were observed in control animals. The incidence and severity of the heart changes increased with the level of erucic acid, especially at doses above 2.0 g/kg bw per day. Fibrosis was already observed at the lowest dose of erucic acid. The CONTAM Panel identified a LOAEL of 1.0 g/kg bw per day based on myocardial lipidosis and fibrosis.

From the identified studies, the CONTAM Panel concluded that the lowest NOAEL for lipidosis was 0.7 g/kg bw per day in a 7 day feeding study in young male Sprague–Dawley rats reported by Kramer et al. (1992). The CONTAM Panel noted that this NOAEL is below the doses causing non-cardiac effects.

Table 11: Identified repeated dose toxicity studies in rats exposed to high erucic acid rapeseed oils via the diet

Duration	Age of the animals/sex/strain[c] (number of animals/group)	Percentage (w/w) and type of oils/fats/lipids in the diet[f]	Dose (g EA/kg bw per day)[d]	NOAEL (g EA/kg bw per day)	LOAEL (g EA/kg bw per day)	Critical effects	Reference
7 days	Males (Sprague–Dawley) (10/group)	EA-oils[e]: • 20% corn oil (0% EA), 20% RSO mixtures (2.9% or 10.1% EA) or 20% HEAR (42.9% EA) in combination with low saturated fatty acids • In addition, 2 oils with 2.4% and 8.7% EA were tested but in combination with high saturated fatty acids	0, 0.6–0.7, 2.1–2.4, 10.3[a]	0.7	2.1	Weight gain: decrease in group receiving 10.3 g/kg bw per day **Myocardial lipidosis**: signs in the corn oil group, significant increase in lipidosis in rats receiving 2.1 g/kg bw per day + accumulation of EA in lipids, and extensive lipidosis in group receiving 10.3 g/kg bw per day + increase in cardiac triacylglycerol Incidence: 8/10 & 10/10, 10/10 & 10/10 and 10/10 Severity (area %): 14 ± 5.6 & 13 ± 4.8, 56 ± 9.0 & 44 ± 5.6 and 100% High level EA in cardiac triacylglycerol and free fatty acids, similar incorporation into phosphatidylserine, followed by sphingomyelin	Kramer et al. (1992)
7 days	Male Weanling (15/group)	EA-oils: 0%, 2.5%, 5%, 10%, 15% or 20% RSO (29.7% EA) or 10% or 20% canbra oil (currently referred to as LEAR; 2.9% EA) (total fat content in diet was 20%)	0, 0.3, 0.7, 0.9, 1.8, 3.6, 5.3, 7.1[a]	1.8 (deposition of total fatty acid and erucic acid in the heart) 3.6 (lipidosis)	3.6 (deposition of total fatty acid and erucic acid in the heart) 7.1 (lipidosis)	Heart: increase deposition total fatty acid and EA from 3.6 g/kg bw per day At 7.1 g/kg bw per day deposition of fat globules within myocardial cells (**myocardial lipidosis**) and between the myofibrils Other changes at 7.1 g/kg bw per day: interstitial oedema, myocytolytic changes in the cardiac muscle and some focal areas of **necrosis**	Beare-Rogers et al. (1971)

Duration	Age of the animals/sex/ strain[c] (number of animals/ group)	Percentage (w/w) and type of oils/ fats/lipids in the diet[f]	Dose (g EA/kg bw per day)[d]	NOAEL (g EA/kg bw per day)	LOAEL (g EA/kg bw per day)	Critical effects	Reference
7 days	Male(40/group) Sprague–Dawley rats 3 weeks of age	Low-fat diet (5% fat) for 14 weeks followed by EA oils for 1 week: 20% corn oil (0% EA), or 20% RSO (1%, 10%, 20%, 30%, 40% or 50% EA) +/– Return to low-fat diet for 1 week	0, 0.1, 1.6, 4.1, 5.2, 7.0 and 8.8[c]	1.6	4.1	Growth depression and reduced feed intake in rats fed diets rich in EA. Growth depression effect of EA disappeared on return to low-fat diet **Myocardial lipidosis**: dose-related increase in severity, reversible after return to low-fat diet without EA Relative lipidosis grading: no fat stain, very slight, slight, moderate, moderate and marked **Myocardial necrosis**: dose-related increase in incidence (not reported for control, 7/30, 9/30, 17/30, 15/30, 9/30 and 16/30) not in severity (results not linear) Cardiac phospholipids: higher concentration	Kramer et al. (1988)
2 weeks	3-week-old male (Wistar) (12/group)	EA-oils: 0%, 5%, 10%, 15%, 20%, 25% and 30% RSO (46% EA) (total fat content not reported)	0, 2.6, 5.5, 8.8, 12.6, 17.0 or 22.1[a]	2.6	5.5	Growth rate: slightly increased as the RSO content of the diet raised from 5% to 10% but decreased with further increases **Adrenals**: hypertrophy of cortical cells at ≥ 12.6 g/kg bw per day **Myocardial lipidosis** fatty infiltration from 5.5 g/kg bw per day (dose-related increase in severity) **Skeletal muscles**: fatty infiltration from 5.5 g/kg bw per day (dose-related increase in severity)	Abdellatif and Vles (1970a)

Duration	Age of the animals/sex/ strain[c] (number of animals/ group)	Percentage (w/w) and type of oils/ fats/lipids in the diet[f]	Dose (g EA/kg bw per day)[d]	NOAEL (g EA/kg bw per day)	LOAEL (g EA/kg bw per day)	Critical effects	Reference
1 or 16 weeks	Weanling males (COBS®) (10–12/group)	EA-oils: 0%, 2.5%, 5%, 10%, 15% or 20% RSO (38.1% EA) Other oils: 0–15% partially hydrogenated RSO (35.2% 22:1) or 0–15% PHHO (31.3% C22:1) (total fat content in diet was 20%)	0; 0.9; 1.7; 3.4; 5.1; 6.9[b]	0.9 (necrosis and fibrosis) 1.7 (lipidosis)	1.7 (necrosis and fibrosis) 3.4 (lipidosis)	Heart: accumulation of lipids (**myocardial lipidosis**) mainly in triacylglycerols (high concentration of 22:1). Increased fatty acids after 1 week after which it regressed Hearts positive for fat stain were: 0/10, 0/10, 0/10, 5/10, 9/10 and 10/10 **Necrosis and fibrosis** at week 16 Incidence of lesions in RSO rats at week 16: 0/12, 0/12, 3/12, 3/12, 6/12, 9/11. Similar incidences of lesions were observed in rats fed with partially hydrogenated RSO or partially hydrogenated herring oil	Beare-Rogers et al. (1972b)

Duration	Age of the animals/sex/ strain[c] (number of animals/ group)	Percentage (w/w) and type of oils/ fats/lipids in the diet[f]	Dose (g EA/kg bw per day)[d]	NOAEL (g EA/kg bw per day)	LOAEL (g EA/kg bw per day)	Critical effects	Reference
32 weeks Sacrifices: day 3, 6, and week 32	3-week-old (Sprague–Dawley) (24/group)	EA-oils: 0%, 2.5%, 5%, 7.5%, 10%, 12.5% or 15% RSO; levels of EA in dietary fat: 0%, 5.5%, 11.0%, 16.5%, 22.0%, 27.5%, 33.0% (total fat content in diet was 20%)	0, 1.0, 2.0, 3.0, 4.0, 5.0, 5.9[b]	–	1.0	Growth rate: lower in rats receiving 5.9 g/kg bw per day **Myocardial lipidosis**: dose-related; observed in all groups receiving RSO after 3 and 6 days and decreased thereafter **Fibrosis** observed after 32 weeks, already in rats receiving 1.0 g/kg bw per day. Dose-related increased incidence and severity of fibrosis. Mononuclear cell proliferation Heart mild cardiopathy: 2/7, 3/8, 5/8, 2/8, 3/7, 2/8, 4/8 Definite or severe cardiopathy: 0/7, 1/8, 0/8, 3/8, 3/7, 6/8, 3/8 **Skeletal muscle, diaphragm**: lipidosis observed after 3 and 6 days **Kidneys**: slight tubular dilatation, increased debris in the lumina of renal tubules after 32 weeks (specially in group fed 15% RSO) **Adrenals**: lipidosis observed after 3 and 6 days. Dose-related enlargement of cortical cells from 5% RSO after 32 weeks	Abdellatif and Vles (1973a)

bw: body weight; EA: erucic acid; HEAR: high erucic acid rapeseed oil; LEAR: low erucic acid rapeseed oil; LOAEL: lowest observed adverse effect level; NOAEL: no observed adverse effect level; PHHO: partially hydrogenated herring oil; RSO: rapeseed oil.
(a): Dose calculated using a default factor of 0.12 for a subacute study in rats (EFSA Scientific Committee, 2012).
(b): Dose calculated using a default factor of 0.09 for a subchronic study in rats (EFSA Scientific Committee, 2012).
(c): If reported by the authors.
(d): Doses are only reported for oils for which the level of erucic acid is known.
(e): Oils for which it can be assumed that all 22:1 present is erucic acid or that do not contain 22:1.
(f): When fat percentage was reported as cal%, the CONTAM Panel recalculated the data as weight% by using a factor of 2.

Pigs

The studies in pigs with high erucic acid oils or studies with both high and low erucic acid oils are described in Appendix G Table G.2. The text below summarises the toxic effects observed in these studies.

In pigs, the heart is also the principal target organ of toxicity following exposure to erucic acid. A dose–response relationship between the level of erucic acid in the diet and the severity of myocardial lipidosis has been observed (Opstvedt et al., 1979; Kramer et al., 1990). The severity of lipidosis was higher in newborn than in weaned pigs. Kramer et al. (1990) suggested that the immature myocardium may be less able to metabolise long-chain fatty acids, making neonates especially prone to myocardial lipidosis, albeit transiently.

Foci of myolysis or necrosis with macrophage or leukocyte infiltration were observed in hearts of pigs fed HEAR oil, other vegetable oils and marine oils (Friend et al., 1975, 1976; Svaar et al., 1980). However, incidence and severity of necrosis were independent of the erucic acid concentration and type of oil diet. In contrast, these lesions were not observed when using a conventional diet (e.g. standard chow or diet containing lard), indicating that diets containing high oil levels are not well tolerated by pigs. Increased total mitochondrial volume due to an increase in number and size of mitochondria was reported in male pigs fed rapeseed oil for more than 30 days, followed by degenerative changes after 60 days feeding and even complete disappearance (Vodovar et al., 1977).

Haematological effects (decrease in platelets counts, increased platelet volume, higher bleeding time) were reported in newborn Yorkshire piglets exposed for 4 weeks to HEAR oil (Kramer et al., 1998). Liver morphological alterations such as increased number of tortuous cisternae of rough surfaced endoplasmic reticulum, signet ring-shaped mitochondria and cytoplasmic lacunae were observed in pigs fed rapeseed oil. Bile canalicular lamina occluded by swollen microvilli and/or globules of a lipid-like material were also seen. These changes represent functional modifications of hepatocytic metabolism in response to oil supplementation (Friesen and Singh, 1981), and therefore, they are very unlikely to be due to erucic acid.

Based on these observations, the CONTAM Panel considered that lipidosis is a relevant effect for the risk assessment of erucic acid.

Studies relevant for hazard characterisation

Due to the high number of studies available (see Appendix G, Table G.2), the CONTAM Panel defined four criteria for the identification of studies that are suitable for hazard characterisation; namely

- testing of several doses with a wide dose-range;
- erucic acid being reported as the main source of variation in fatty acid composition of the diet;
- possible identification of a NOAEL;
- observation of myocardial lipidosis as relevant toxic effect.

From the studies described in Appendix G, Table G.2, the CONTAM Panel identified one study that met these criteria. The identified study is summarised in Table 12 and is described in more detail below.

Kramer et al. (1990) fed newborn Yorkshire male and female piglets sow milk (corresponding to an erucic acid dose of 0.09 g/kg bw per day) or milk replacer diets containing rapeseed oil with different levels of erucic acid or soybean oil from birth to 2 weeks of age. Dietary oils with 2.3%, 4.7%, 7.0%, 11.7% and 20.7% erucic acid were prepared by mixing appropriate amounts of canola (0.8% erucic acid) and HEAR (42.9% erucic acid) oils. Based on the information provided by the authors, the CONTAM Panel calculated doses of 0, 0.1, 0.3, 0.7, 1.1, 1.8, 3.0 or 5.1 g/kg bw per day. Animals were initially fasted for 8 h and then fed every 2 h at the rate of solid intake equivalent to 7% of body weight per day. Piglets have been successfully reared with milk replacers; they grew as well as piglets left with the sow. There were no significant diet effects on body weight. Newborn piglets showed no myocardial lipidosis, but lipidosis appeared with consumption of sow milk and disappeared by 7 days of age. Milk replacer diets containing soybean oil or rapeseed oil mixtures with up to 4.7% erucic acid in the oil (doses up to 0.7 g/kg bw per day) gave trace myocardial lipidosis. Doses of 1.1 g/kg bw per day or more caused definite dose-related myocardial lipidosis in newborn piglets, with a maximum after 1 week on diet. This may be related to the low capacity of the fetus to oxidise fatty acids which increased rapidly after birth (Werner et al., 1983). The authors indicated that the severity of the lipidosis was greater than observed previously by Opstvedt et al. (1979) and Kramer et al. (1975) with weaned pigs. Focal myocardial necrosis was not observed in any of the piglets fed sow milk or any of the milk replacer diets. There were no significant differences among diets in cardiac lipid classes except for triacylglycerol which increased in piglets fed rapeseed oil with the highest content of erucic acid. The CONTAM Panel identified a NOAEL of 0.7 g/kg bw per day based on lipidosis.

Table 12: Identified repeated dose oral toxicity study in pigs

Duration	Sex/age of the animals/species (number of animals/group)	Percentage (w/w) and type of oils/fats/lipids in the diet[a]	Dose (g EA/kg bw per day)	NOAEL (g EA/kg bw per day)	LOAEL (g EA/kg bw per day)	Critical effects	Reference
Birth to 14 days	Newborn male and female Yorkshire piglets (2–20/group)	EA oils[a]: 25% SBO (0% EA) or 25% RSO (0.8%, 2.3%, 4.7%, 7.0%, 11.7%, 20.7% or 42.9% EA) Or sow milk (0.1% EA)	0; 0.1; 0.3; 0.7; 1.1; 1.8; 3.0; 5.1[c] 0.09[b]	0.7	1.1	**Myocardial lipidosis** in piglets fed sow milk (0.09 g EA/kg bw per day), disappearing by day 7. Trace myocardial lipidosis in piglets fed SBO or RSO up to 0.7 g EA/kg bw per day. Definite dose-related lipidosis in piglets receiving ≥ 1.1 mg/kg bw per day, with a maximum after 1 week diet Severity of lipidosis was higher in newborn than in weaned pigs **Necrosis** was not observed	Kramer et al. (1990)

bw: body weight; EA: erucic acid; LOAEL: low-observed-adverse-effect level; NOAEL: no observed adverse effect level; RSO: rapeseed oil; SBO: soybean oil.
(a): Oils for which it can be assumed that all 22:1 present is erucic acid or that do not contain 22:1.
(b): Dose from sow milk. Because of the difficulties associated with measuring milk consumption by pre-weaned piglets, few estimates are available. However, based on the studies of Theil et al. (2002) and Aguinaga et al. (2011), the CONTAM Panel assumed a milk intake of 280 g/day. From a figure in the paper, the CONTAM Panel derived a mean body weight of 3.25 kg.
(c): Mean doses calculated from the doses provided by the authors for the different ages of the animals.

In summary, increased myocardial lipidosis is observed in newborn pigs at erucic acid doses of 1.1 g/kg bw per day and the CONTAM Panel identified a NOAEL of 0.7 g/kg bw per day for this endpoint.

Monkeys

The studies in monkeys with high erucic acid oils or studies with both high and low erucic acid oils are described in Appendix G Table G.3. The text below summarises the toxic effects observed in these studies.

Pronounced myocardium lipidosis as well as increased size of mitochondria were observed in monkeys exposed to HEAR oil and to partially hydrogenated herring oil (e.g. Ackman and Loew, 1977; Loew et al., 1978; Schiefer et al., 1978). In some studies, foci of mononuclear cell infiltration were observed, but they were not specific to animals fed HEAR oil. Lipidosis was also observed in skeletal muscles. The erucic acid level in heart total lipids and in triacylglycerols increased in monkeys exposed to HEAR oil. There was no indication of gross myocardial changes indicative of myocardial enlargement, cardiac failure or myocardial ischemia. No adverse effect was observed on blood coagulation. No myocardial damage was associated with feeding rapeseed oil. In a study where adult male monkeys were fed diets containing mustard oil for 1 year, sarcoplasmic vacuolation of right and left ventricular myocardium was shown as well as myocardial fibrosis (Gopalan et al., 1974). The relevance of these studies is limited due to the low number of doses and animals tested.

From the studies described in Appendix G, Table G.3, no study was identified in which a wider dose-range was studied. Therefore, none of the studies were considered relevant for the risk assessment of erucic acid.

Other animal species

The studies in mice, rabbits and gerbils with high erucic acid oils or studies with both high and low erucic acid oils are described in Appendix G Table G.4. The text below summarises the toxic effects observed in these studies.

In mice, a significant increase in the heart weight and liver was observed in animals fed rapeseed oil containing high levels of erucic acid for 5 weeks (Lei et al., 2010). Long-term exposure of rabbits to high levels of erucic acid resulted in increases in total serum lipids, cardiac interstitial fibrosis associated with vacuolar changes and disarrangement and dissociation of the myofibrils. In the liver, portal fibrosis (cirrhosis) was seen, sometimes associated with hyperplasia of the bile ducts and/or the ductular cells. Atherosclelerotic changes were also observed in the thoracic aorta of some rabbits (Abdellatif and Vles, 1971b). After 1 week feeding of gerbils, more cardiac fatty acids deposits including erucic acid were observed in animals fed high erucic acid levels than in control animals or animals fed a low erucic acid level. Young gerbils were particularly susceptible to the deposition of fatty acids from rapeseed oil (Beare-Rogers and Nera, 1972).

From the studies described in Appendix G, Table G.4, no study was identified in which a wide dose-range was studied. Therefore, none of the studies were considered relevant for the risk assessment of erucic acid.

Summary

The heart is the principal target organ following short-term or long-term exposure of rats, pigs, monkeys, rabbits and gerbils to diets with oils containing erucic acid. The most common and sensitive effect observed in all species is myocardial lipidosis. Lipidosis is transient and reversible: it develops very quickly (a few hours after starting exposure), reaches a peak after 3–7 days and regresses thereafter, even during continued feeding of high erucic acid diets. Species differences appear to exist with respect to responses to erucic acid-containing oils. Studies in rats and, newborn and weanling pigs showed an association between the level of erucic acid in the diet and the severity of myocardial lipidosis. In rats, increased myocardial lipidosis is observed at doses of 1 g erucic acid/kg bw per day or higher. In newborn pigs, increased myocardial lipidosis is observed at a dose of 1.1 g erucic acid/kg bw per day. The overall NOAEL for lipidosis was 0.7 g/kg bw per day in a 7-day feeding study in young rats and in a 2-week feeding study in newborn piglets. Adult pigs are able to tolerate higher levels of erucic acid than young animals. These results suggest that the immature myocardium and/or liver may be less able to metabolise erucic acid, making neonates especially prone to myocardial lipidosis, albeit transiently. Myocardial lipidosis is reported to reduce the contractile force of the heart muscle. Mitochondrial damage (megamitochondria, mitochondrial proliferation, increase in the average volume,

distortion of shape, degeneration) and disorganisation of myofibrils has been reported after exposure of rats, pigs, monkeys or rabbits to high doses of erucic acid.

Myocardial necrosis and fibrosis have been observed in rats and occasionally in other species after erucic acid exposure via rapeseed oils for 4 or more weeks. However, these effects were also observed in animals fed oils not containing erucic acid and the incidence and severity were independent of the erucic acid dose and the type of oil diet. Myocardial necrosis in rats is influenced by many factors such as age, sex, strain and breeding source. The results of several studies suggest that factors other than, or in addition to, erucic acid are responsible for the increased incidence of lesions observed in rats fed with oils high in erucic acid, e.g. fatty acid imbalance. Therefore, the CONTAM Panel considered necrosis observed in these studies not a suitable endpoint for the risk assessment. A causal link between myocardial lipidosis and myocardial lesions has not been established.

3.3.2.3. Genotoxicity

No genotoxicity studies on erucic acid were identified in the literature. Negative results in *in vitro* genotoxicity assays (Ames test in *Salmonella* Typhimurium and DNA damage and repair assays in *Bacillus subtilis*) are claimed for erucic acid in a compilation of data from the European Chemicals Bureau (European Commission, 2000). One of the study reports was made available by the data owner (Henkel KGaA, 1981b). However, because of the lack of raw data these studies cannot be used for the evaluation of erucic acid genotoxicity. Therefore, no conclusion can be drawn on its genotoxic potential.

3.3.2.4. Carcinogenicity

In general, it is difficult to identify the effect of individual fatty acids e.g. on cancer incidence, as the diet always contains a complex natural fat or oil as the source of fatty acids. Experimental studies on mice have been conducted to clarify the role of main fatty acids by selecting different fats, oils and their mixtures in order to have sufficiently wide ranges for individual fatty acids in 20 different diets (Tinsley et al., 1981), which were given to mice for 27 weeks. Statistical methods were used to further isolate the effects of individual fatty acids on the incidence and development of mammary tumours in C3H mice. The results indicated that most fatty acids have little effect on tumour incidence. Increased incidences were found for linoleic acid (18:2 n-6) and stearic acid (18:0). In this experimental setting, there was a suggestion that erucic acid reduced tumour incidence but it cannot be excluded that this effect could be due to some other constituent of the rapeseed oil.

Carroll and Khor (1971) reported a promoting effect on 7,12-dimethyl-benz[a]anthracene-induced mammary adenocarcinomas when rats were kept on a diet containing 10–20% corn oil. Experiments were conducted with different fats and oils including rapeseed oil (34.5%) fed at the 20% level (corresponding to an erucic acid dose of 6.2 g/kg bw per day using a default factor of 0.09 (EFSA Scientific Committee, 2012)). In general more tumours per rat were observed when unsaturated fats were fed. Rapeseed oil was the exception and the low yield of tumours with this oil was explained by the authors as likely related to the high content of 20:1 and 22:1 fatty acids.

In summary, the studies currently available do not allow drawing conclusions on the carcinogenicity of erucic acid.

3.3.2.5. Developmental and reproductive toxicity

In the study by Carroll and Noble (1957) male rats were given a diet of powdered Master Meal with a supplement of 10% or 15% by weight of erucic acid or 25% rapeseed oil (containing approximately 50% by weight of erucic acid in the triacylglycerols). Based on the available information and using a default factor of 0.09 (EFSA Scientific Committee, 2012), the CONTAM Panel calculated erucic acid doses of 9.0 and 13.5 g/kg bw per day for the erucic acid groups and 11.3 g/kg bw per day for the rapeseed oil group. The fertility of the animals was monitored and, after different periods on the diet, animals were killed and the testes, seminal vesicles and prostate were analysed at autopsy. The fertility of the males receiving 13.5 g/kg bw per day for approximately 3 months was markedly affected and after 5 months, mating rarely occurred. No effects on fertility were observed when the feeding was with diet supplemented with 15% oleic acid or 25% rapeseed oil (11.3 g/kg bw per day). The authors hypothesise that the presence of other fatty acids in the rapeseed oil might have moderated the effects of erucic acid. The weight of the testes was markedly increased in the growing animals fed with the erucic acid-supplemented diet while no effects were observed in adult animals maintained with the same diet. The weight of the seminal vesicles and prostate appeared normal. Histological alterations were observed in the spermatogenic cells of the testes with increasing

degenerative changes after 3 months on the erucic acid-supplemented diets. Conversely, no changes were observed in the tubules of adult rats fed with this diet for 4 months. The morphological changes were permanent and irreversible. Moreover, they were confined to the tubular epithelium and were not associated with alterations of the Leydig cells neither with effects on male sex hormones secretion. Since the testis is rich in highly unsaturated fatty acids, the erucic acid may act by competing with fatty acids essential for normal testicular function, however on the basis of the available data the mechanism remains speculative. In the same study the feeding of the erucic acid supplement to the mother for more than 8 weeks resulted in poor survival of the young but since the supplement with oleic acid showed the same effect this cannot be considered specific for erucic acid. The animals which died after parturition or were killed showed only occasionally changes in endocrine organs. As in the case of the male rats prolonged feeding caused impairment of the reproductive process.

In a following study Beare et al. (1959) were unable to reproduce the effects on spermatogenesis reported by Carroll and Noble (1957). Male Wistar rats fed for 9 weeks a diet containing 20% Golden rapeseed oil (42.7% erucic acid in the oil; equivalent to a dose 7.7 g erucic acid/kg bw per day using the default factor of 0.09 for a subchronic study (EFSA Scientific Committee, 2012)), successfully sired 3 L and showed no abnormalities when scarified at 11 months of age. However the authors noted that in their experimental setting the diet administered to rats contained a significant fraction of linoleic acid and a lower level of erucic acid than that employed by Carroll and Noble (1957) where erucic acid was the only fatty acid added to a low-fat diet.

In the study by Rose and Bell (1982) female Swiss mice were fed a purified diet containing 20% of different types of fats and oil. The tested fats and oils were lard/corn oil (0% erucic acid), LEAR (0.1% erucic acid), stinkweed oil (41% erucic acid) and screening oils with different erucic acid content (2.8%, 5.8% and 5.2%). Based on the available information and using a default factor of 0.2 (EFSA Scientific Committee, 2012), the CONTAM Panel calculated erucic acid doses of 0, 0.04, 1.1, 2.1, 2.3 and 16.4 g/kg bw per day. This diet was given for a 2-week preliminary period and then during 18 days of gestation. No evidence of teratogenic effects was reported when LEAR oil (0.04 g/kg bw per day) was incorporated into the diet. Feed consumption levels were reduced in some cases, perhaps due to reduced palatability, thus accounting for decreased reproductive performance. Mice fed the LEAR diet had a decreased incidence of cleft palate in their fetuses as compared with mice fed the lard:corn oil control diet.

In the study by Reyes et al. (1995) pregnant rats and hamsters were fed for 90 days prior to mating and until the last day of pregnancy with diets containing either 25% rapeseed oil rich in erucic acid (41.5%) or corn oil (0.5% erucic acid). Based on the available information and using a default factor of 0.09 (EFSA Scientific Committee, 2012), the CONTAM Panel calculated erucic acid doses of 0.1 and 9.3 g/kg bw per day. Mating was successful for both rats and hamsters. The number and weight of fetuses were similar when animals were fed either rapeseed or corn oil. No morphological abnormalities were observed in any of the fetuses. Histological examination conducted the last day of pregnancy (day 20 for rats and day 14 for hamsters) showed no morphological abnormalities in the liver, heart, kidneys and adrenals. Mild myocardial lipidosis was observed in pregnant hamsters fed both oils. The dietary profile was reflected in the fatty acid composition of the liver, heart and kidney. Erucic acid was found in the highest proportion in the heart (14%) of animals fed rapeseed oil. Pregnancy induced changes in bile secretion in rats and hamsters irrespective of the diet administered.

In summary, no major adverse reproductive and developmental effects were associated with feeding female rats, mice and hamsters with erucic acid-containing diets prior to mating and during pregnancy. There is one limited report of affected fertility of male rats maintained under prolonged feeding with an erucic acid-supplemented diet but since these observations were made only in one study and one species (i.e. rat), the results should be confirmed in order to be used in risk assessment.

3.3.3. Observations in humans

Few studies have determined esterified erucic acid levels (in phospholipids and neutral lipids) in human plasma, however no studies were identified that discriminate between albumin bound and unbound fractions of unesterified fatty acids.

3.3.3.1. Biomonitoring studies

The NHANES food database reports mean serum concentrations for esterified erucic acid of 3.4 μmol/L (95% CI: 2.77–3.79) in a 1,845 sample subset of the large cohort.[39]

In a recent report, average concentrations of 61 fatty acids including esterified erucic acid in plasma of young (age ranging from 20 to 29 years), healthy and ethnoculturally diverse Canadians consuming their usual diet were studied. Participants were recruited between September 2004 and July 2009. The authors found mean plasma erucic acid levels of 3.9 μmol/L (range: trace – 48.0 μmol/L). The study was part of the cross-sectional Toronto Nutrigenomics and Health Study. The total population studied was 826 (327 males and 499 females) (Abdelmagid et al., 2015).

3.3.3.2. Epidemiological studies

Epidemiological studies on potential adverse effects of dietary erucic acid intake are scarce and only a limited number of independent studies were identified. These studies are however, large case–control studies. Negative controls were employed to estimate potential bias.

Cardiovascular effects

Imamura et al. (2013) studied the association between plasma phospholipid long-chain monounsaturated fatty acids (LCMUFAs) (20:1, 22:1 and 24:1), used as biomarker of exposure, and the incidence of congestive heart failure. This association was studied in two independent cohorts; the Cardiovascular Health Study (CHS; 3,694 subjects; mean age 75.2 ± 5.2 years) and the Atherosclerosis Risk in Communities Study (ARIC; 3,577 subjects; mean age 54.1 ± 5.8 years). To determine whether the associations were myocardium specific, incident stroke was used as a negative control outcome. In addition, the authors evaluated intake levels based on consumption data obtained in the NHANES 2003–2010 study (Ervin et al., 2004; U.S. Department of Agriculture - Agricultural Research Service[40]) and on dietary consumption data collected in both CHS and ARIC cohorts.

The level of 22:1 plasma phospholipids (expressed as per cent of total fatty acids) were 0.03 ± 0.01% and 0.01 ± 0.03% in the CHS and ARIC cohorts, respectively. These levels were much lower than the levels of 24:1 which were 1.96 ± 0.44% and 0.57 ± 0.17% in the CHS and ARIC cohorts, respectively. The level of 22:1 was below the LOD (0.01%) in 43% of the subjects of the ARIC cohort.

In both cohorts, higher circulating levels of 22:1 and 24:1 plasma phospholipids were positively associated with an increased incidence of congestive heart failure, which suggests the possible cardiotoxicity of LCMUFAs in humans. Since dietary 22:1 is elongated to 24:1 in humans, the authors suggested that the experimentally observed cardiotoxicity from 22:1 could be partly attributed to 24:1. Many different foods contribute to the LCMUFA exposure and the authors concluded that the potential cardiotoxicity of LCMUFAs cannot be attributed to any single food but depends on the overall exposure to LCMUFAs.

Matsumoto et al. (2013) studied the association between coronary artery disease (CAD) and red blood cell monounsaturated fatty acids (MUFA) levels. CAD is characterised by the accumulation of plaques in the coronary artery, which finally occlude the artery resulting in coronary heart disease (CHD). In the study by Matsumoto et al., CAD was defined as a non-fatal myocardial infarction, fatal myocardial infarction, percutaneous transluminal coronary angioplasty, coronary artery bypass graft, coronary death and sudden death.

A prospective, nested, case–control study was performed in which 1,000 cases of incident CAD and 1,000 control subjects matching age, year of birth and time of blood collection were selected from a cohort of US male physicians (the Physicians' health study). The concentrations of different monounsaturated fatty acids were measured from erythrocytes. All cardiovascular events in the Physicians' health study have been adjudicated by an endpoint committee. The diagnosis of myocardial infarction was confirmed by using WHO criteria. Revascularisation procedures were confirmed by hospital records.

The authors identified an inverse association between red blood cell 22:1 n-9 levels and CAD after adjustment for multiple comparisons (odds ratio: 0.83 (95% confidence interval: 0.72, 0.95; p = 0.0086). Red blood cell *cis* 18:1 n-9 and 24:1 n-9 levels were not associated with CAD risk.

[39] http://www.cdc.gov/nchs/nhanes.htm/

[40] U.S. Department of Agriculture, Agricultural Research Service. 2009. USDA National Nutrient Database for Standard Reference, Release 22. Nutrient Data Laboratory Home Page. http://www.ars.usda.gov/ba/bhnrc/ndl

Based on the study by Imamura et al. (2013) it can be concluded that higher levels of 22:1 fatty acids in plasma phospholipids have been associated with higher incidence of congestive heart failure in two independent cohorts. In another cohort, higher circulating levels of erucic acid in erythrocytes have been associated with lower incidence of coronary heart disease (Matsumoto et al., 2013).

Cancer

Sczaniecka et al. (2012) studied the association between breast cancer and dietary intake of specific fatty acids in post-menopausal women. The participants were female members of the VITamins And Lifestyle Cohort, previously described by White et al. (2004). After applying specific exclusion criteria, 30,252 women entered the study; 772 cases and 29,480 controls. Dietary consumption data were obtained by semi quantitative food frequency questionnaire specifically modified to facilitate the fatty acid intake estimations. During the years 2000–2002, study subjects reported usual frequency and portion size of 120 foods and beverages consumed during the year before baseline with the help of photographs of portion sizes. The consumption data were converted to intake of specific fatty acids by using Minnesota Nutrient Data System for Research.[41]

The intake of total MUFAs (highest vs. lowest quintile hazard ratio: 1.61, 95% confidence interval: 1.08–2.38, p = 0.02) was associated with an increased breast cancer risk. The CONTAM Panel noted that for erucic acid, both an association with a reduced and an increased breast cancer risk was reported.

The authors concluded that 'although further study of specific fatty acids is needed, this study provides further support for the hypothesis that fat consumption is associated with breast cancer risk and suggests that risk varies by type of fatty acid'. From this study, the CONTAM Panel concluded that the evidence is not strong enough to draw conclusions regarding the association between erucic acid and breast cancer. The estimation of the erucic acid exposure was based on a food frequency questionnaire but no biomarker for exposure was used.

Ukoli et al. (2010) studied the association between prostate cancer and dietary fat using a case–control study. Two genetically related populations were included, namely African Americans (48 cases and 96 controls) and Africans (66 cases and 226 controls).

Significant differences were observed for 13 out of 21 circulating fatty acids in cases compared to the controls in both populations. No association was found between the level of erucic acid and the risk of prostate cancer in Africans (odds ratio = 1.06 (95% confidence interval: 0.52–2.16); adjusted odds ratio = 0.94 (95% confidence interval: 0.40–2.20)), however an association was shown in African Americans (odds ratio = 2.95 (95% confidence interval: 1.01–8.60); adjusted odds ratio = 3.96 (95% confidence interval: 1.05–14.9)). Also for other fatty acids (e.g. DHA, nervonic acid and arachidonic acid (20:4 n-6)) such an association was observed. The association between plasma fatty acid profiles and prostate cancer is therefore not specific to erucic acid.

In summary, two studies on the possible association between cancer and erucic acid exposure were identified but no conclusion can be drawn due to the intrinsic limitations or lack of specificity of the outcome.

3.3.3.3. Adverse effects due to therapeutic use

Lorenzo's oil is an erucic acid-containing drug used to treat patients with ALD, which is an inherited, X-linked, peroxisomal disease (OMIM database 300100[42]) leading to total disability by motor deficits, dementia, impaired vision and hearing (Moser, 1997). Characteristic to the disease are myelopathy, peripheral neuropathy and cerebral demyelopathy. In ALD patients, the genetic defect resides in the *ABCD1* gene encoding for a peroxisomal membrane transporter for very long-chain fatty acids (Mosser et al., 1993; van Roermund et al., 2011). ALD is the only disease associated with deficiency of a peroxisomal ABC transporter. Diagnosis of ALD is confirmed by increased levels of very long-chain fatty acids (> 20 carbon chain length) in the circulation.

Lorenzo's oil inhibits the activity of the enzyme ELOVL1 (elongation of very long-chain fatty acid), the primary enzyme responsible for the synthesis of saturated and monosaturated very long-chain fatty acids (VLCFAs), by a mixed inhibition mechanism, rather than by competitive inhibition. Lorenzo's oil reduce the saturated VLCFA level in the plasma of X-ALD patients. The optimal inhibition requires both erucic acid and oleic acid, as erucic acid alone does not inhibit ELOVL1 significantly (Sassa et al., 2014).

[41] http://www.ncc.umn.edu/products/ndsr.html
[42] http://www.omim.org

The clinical use of the drug has been reported to be beneficial to presymptomatic patients, but has very minor effect if administered after onset of the disease (Berger and Gärtner, 2006).

Doses of Lorenzo's oil are expressed, depending on the publication, in different ways. Rasmussen et al. (1994) reported typical intakes of 2.5–4 tablespoons (15 mL/tablespoon) per day. This is equivalent to 0.09–0.14 g erucic acid/kg bw per day assuming a body weight of 70 kg and 0.27–0.44 g erucic acid/kg bw per day assuming a body weight of 23 kg for children between 3 and 10 years old.[43] In the study by Deon et al. (2006) the patients were treated with a higher dose of 2–3 mL of Lorenzo's oil/kg bw per day (adjusted individually) corresponding to 0.34–0.51 g erucic acid/kg bw per day.[44] Many authors e.g. Zinkham et al. (1993) expressed the dose as a percentage of the daily caloric intake, typically 20%. A dose of 20% of caloric intake is estimated to correspond approximately to 2–3 mL/kg bw per day of Lorenzo's oil, i.e. 0.4–0.6 g/kg bw per day of erucic acid (Moser et al., 2005).

The therapeutic uses of Lorenzo's oil have been reported to induce haematological effects. In a clinical trial, ALD patients received either no supplemental oil (n = 13), only glycerol trioleate (45–90 mL per day) (n = 12) or Lorenzo's oil (37.5 to 60 mL/day corresponding to an erucic acid dose of 0.09–0.14 g/kg bw per day as calculated above) (n = 13) for 6 months. In addition, the results were compared with 33 healthy controls, which were on their customary diet. The group receiving Lorenzo's oil showed a statistically significant decrease of about 1.5-fold in platelet counts after 6 months. The authors reported a strong inverse relationship between platelet counts, and erucic acid and other n-9 fatty acid levels (Kickler et al., 1996). In addition, megathrombocytes were observed in the group treated with Lorenzo's oil. Appearance of such giant thrombocytes indicates accelerated thrombocyte turnover. Zierz et al. (1993) reported decreased number of platelets (thrombocytopenia) in three of five patients with different phenotypes of ALD treated daily during 1 year with 60–70 g/day of Lorenzo's oil providing 11.6–13.5 g erucic acid/day (equivalent to 0.17–0.19 g/kg bw per day assuming a body weight of 70 kg). In this study the platelet count showed about a 4-fold decrease when exposed to a daily dose of 15 g of Lorenzo's oil for 2 months compared to baseline. One case report described an ALD patient treated with varying doses of Lorenzo's oil (0–20% of daily caloric intake; no indication of the dose on mg/kg bw per day basis), in which the treatment resulted in increases of bleeding time, while platelet function seemed to be normal (Chai et al., 1996). Zinkham et al. (1993) reported data from 17 ALD patients, who had thrombocytopenia resulting from 6 month treatment with Lorenzo's oil. The amount of Lorenzo's oil provided 20% of calculated daily caloric intake and the erucic acid dose was not indicated. The thrombocytopenia persisted still after 12 months after the treatment. No uniform platelet-aggregation patterns were found.

Depression of natural killer cells and lymphocytopenia were studied in 27 ALD patients treated with Lorenzo's oil, in 14 ALD patients without the treatment and 26 healthy individuals. The patients received 20% of their total calories as Lorenzo's oil and the duration of the treatment ranged from 4.5 months to 3.2 years. The dose was not indicated on mg/kg bw or per day basis. While lymphocyte proliferation in response to mitogens (phytohaemagglutinin and concanavalin A) in untreated ALD patients was within normal intervals, it was significantly higher in patients treated with Lorenzo's oil. It was concluded that long-term side-effects on cellular immunoreactivity should be followed in ALD patients treated with Lorenzo's oil (Barmaki Pour et al., 2000).

In summary, the therapeutic use of erucic acid results in haematological effects, most notably thrombocytopenia and morphological alterations of thrombocytes, at doses of about 0.1 g/kg bw per day. It results also in increased lymphocyte reactivity to mitogens. Erucic acid induced lipidosis has not been described in humans.

3.3.4. Mode of action

In all experimental studies with repeated dosing of high erucic acid-containing oils to rats, pigs, monkeys and other experimental animals, effects have most often been observed in the heart and less frequently in the liver and adrenals (see Section A. Studies including only low erucic acid rapeseed oils in experimental animals and Appendix G, Tables G.1, G.2, G.3 and G.4). Thus, the heart appears to be the most susceptible organ for adverse effects of erucic acid.

[43] This calculation is based on the assumption of a density of 0.9 kg/L, a body weight of 70 kg and a table spoon content of about 15 mL. The reported intakes correspond to 34–55 g of Lorenzo's oil per day or 7–11 g glycerol trierucate per day or 6–10 g erucic acid per day.

[44] The reported intakes correspond to 1.8–2.7 g of Lorenzo's oil/kg bw per day or 0.36–0.55 g glycerol trierucate/kg bw per day. This calculation is based on the assumption of a density of 0.9 kg/L.

The major cardiac effects, observed when feeding an erucic acid-containing diet to experimental animals, are the early but transient accumulation of lipid droplets within myocytes, i.e. lipidosis, and the later death of myocardial cells (necrosis) followed by the removal of necrotic cellular debris by macrophages and repair by fibrosis (see Section B. Studies including oils high in erucic acid (> 5%)). Whereas the occurrence of a transient cardiac lipidosis is clearly associated with the dietary exposure to erucic acid, the aetiology of cardiotoxic effects such as necrosis and fibrosis is less clear and may involve factors other than erucic acid, e.g. imbalance of fats or lack of essential nutrients (Sauer and Kramer, 1983a). In humans, thrombocytopenia and morphological alterations of thrombocytes have been observed upon therapeutic use of Lorenzo's oil (see Section 3.3.3.3). Haematological effects have also been observed in experimental animals fed with HEAR oils, e.g. in pigs (Kramer et al., 1998). The mechanism for erucic acid induced thrombocytopenia is not known.

3.3.4.1. Cardiac lipidosis

Both male and female rats of various strains accumulate triacylglycerols within cardiac myocytes as lipid droplets or membrane inclusions when kept on a diet with erucic acid for several days. Myocardial lipidosis involves primarily an increase in triacylglycerols while the levels of phospholipids and cholesterol remain fairly constant (Houtsmuller et al., 1970; Beare-Rogers et al., 1972b). Early studies reported an increase in cardiac free fatty acids, but these findings appear to be an artefact caused by hydrolysis of lipids due to inappropriate extraction techniques (Kramer and Hulan, 1978). The incorporation of erucic acid into cardiac lipids is highest in triacylglycerols and moderate in certain phospholipids such as phosphatidylcholine and phosphatidylethanolamine (Kramer et al., 1979b; Yasuda et al., 1980). Lipidosis develops very quickly (a few hours after starting the exposure), reaches a maximum after 3–7 days (depending on the dose), and decreases or disappears after about 4 weeks in rats even when kept on a high erucic acid diet (Sauer and Kramer, 1983a).

The major reason for the development of cardiac lipidosis is thought to be an imbalance of uptake and utilisation of fatty acids (Sauer and Kramer, 1983b). If the heart is supplied with fatty acids such as palmitic, stearic and oleic acid, as present in common dietary lipids, β-oxidation and ATP production in mitochondria proceeds smoothly as described in Section 3.3.1.3. However, after changing to a high erucic acid diet, the supply of erucic acid to the heart via the blood increases, and the mitochondrial β-oxidation is not able to cope with the high influx for two reasons. Firstly, erucic acid is only poorly β-oxidised in rat heart mitochondria, and secondly, erucic acid inhibits the mitochondrial β-oxidation of other fatty acids (see Section 3.3.1.3). Obviously, β-oxidation in peroxisomes is not sufficient to handle the high level of erucic acid. As a consequence, the influx of free erucic and other fatty acids into the heart exceeds their utilisation for β-oxidation and ATP production. Because elevated levels of free fatty acids are toxic, they are converted to triacylglycerols and stored as lipid droplets in myocytes, giving rise to the observed cardiac lipidosis. Lipidosis, although less pronounced than in the heart, is also observed in other organs of the rat shortly after exposure to erucic acid, but not in the liver, probably because the liver can efficiently export erucic acid-containing triacylglycerols as VLDLs (Bremer and Norum, 1982).

The mode of action for cardiac lipidosis outlined for rats exposed to erucic acid is also consistent with observations made with structurally related fatty acids and with other species of experimental animals. Oils containing other monounsaturated fatty acids with 20 or more carbon atoms also induce transient cardiac lipidosis in rats and are poor substrates for mitochondrial β-oxidation (Sauer and Kramer, 1980).

Based on *in vitro* data, the pig heart appears to be somewhat more efficient in the β-oxidation of erucic acid compared to the rat heart. The primary reason for this is believed to reside in the chain length preference of the mitochondrial β-oxidation system, which is much better defined in rats (sharp drop of oxidation rate if $C > 18$) than in pigs, and to the fact that 22:1 fatty acids inhibit the tricarboxylic acid cycle in heart mitochondria of rats but not of pigs (Buddecke et al., 1976; Osmundsen and Bremer, 1978). Induction of cardiac lipidosis in humans after ingestion of high erucic acid diets has not been reported to date.

As mentioned above, cardiac lipidosis is a reversible and transient phenomenon in rats. It regresses after termination but also upon continuation of the high erucic acid diet. Numerous studies suggest that the major reason for this adaptation resides in the induction of the peroxisomal β-oxidation system of the liver and also in the heart, whereas the mitochondrial β-oxidation systems appear not to be induced in both organs (Sauer and Kramer, 1980; Bremer and Norum, 1982). As a high proportion of the dietary fat (estimated to be at least 50%) passes through the liver, an enhanced hepatic peroxisomal β-oxidation of erucic acid would lower the influx of this fatty acid to the heart and

eventually lead to the regression of cardiac lipidosis, facilitated by the rapid turnover (estimated to be about 5 h) of cardiac triacylglycerols.

Consistent with the concept of peroxisomal induction is the observation that the cardiac lipidosis induced by a high erucic acid diet in young rats is markedly reduced by simultaneous feeding of peroxisome proliferators such as clofibrate (Christiansen et al., 1979). Clofibrate is an agonist of peroxisome proliferator-activated receptor-alpha (PPARα), which together with other PPARs orchestrates many aspects of fatty acid metabolism and storage in the heart and other organs (Hihi et al., 2002).

3.3.4.2. Cardiac effects other than lipidosis

In addition to cardiac lipidosis, cardiac necrosis and fibrosis have been observed in the heart following feeding of high erucic acid diets. As these effects cannot be specifically ascribed to erucic acid (see Section 3.3.2.2), they will not be discussed in more detail.

Dietary exposure to high levels of erucic acid has an effect on cardiac mitochondria. One of the putative molecular targets is cardiolipin, which is an acidic lipid found exclusively in mitochondria. It is localised in the inner membranes and required for the mitochondrial β-oxidation of fatty acids and also for the structural organisation of the respiratory chain (Mileykovskaya and Dowhan, 2014). Cardiolipin has a strong preference for linoleic acid in most mammalian tissues. In rats, dietary supplementation with erucic acid or glycerol trierucate results in accumulation of erucic acid in cardiolipin (Blomstrant and Svensson, 1974; Dewailly et al., 1978). However, it remains to be clarified whether replacement of some of the linoleic acid by erucic acid alters mitochondrial functions.

Mitochondria are dynamic structures with a rapid turnover, having a half-life in normal rat myocardium of about 14 days (Stotland and Gottlieb, 2015). They are not only involved in ATP production but also in specialised functions, e.g. in the regulation of apoptosis. In several experimental studies, substantially enlarged mitochondria ('megamitochondria') have been found in the rat heart after feeding a high erucic acid diet (Bhatnagar and Yamashiro, 1979), which may release cytochrome c with subsequent activation of caspases and initiation of apoptosis.

3.3.4.3. Summary

The major effect observed after erucic acid exposure of experimental animals is transient lipidosis arising mainly from the poor β-oxidation of erucic acid in mitochondria. The reversibility of this effect appears to be due to the enhanced peroxisomal chain shortening mediated by PPARs.

3.3.5. Adverse effects in livestock, fish and companion animals

Many studies have been reported in which the effects of erucic acid intake by farm and companion animals and fish have been examined. The objective of many of the studies has been to examine upper limits to levels of erucic acid-containing feeds – predominantly rapeseed meal – in livestock diets. However, interpretation of the results is difficult because the level of erucic acid has not been reported, and/or because where adverse effects have been reported they may be confounded by the presence of other antinutritive factors in meal, particularly glucosinolates.

3.3.5.1. Ruminants

In common with other livestock, there have been very few studies that have examined specifically the adverse effects of erucic acid *per se*. For most ruminant livestock, rapeseed meal is the main source of exposure to erucic acid, and many studies have been reported in which the effects of replacing other vegetable proteins with rapeseed meal have been examined. From an extensive review of published studies, Hill (1991) concluded that low-glucosinolates rapeseed meal could replace soybean meal with no adverse effect on milk yield or milk composition, a conclusion supported by Huhtanen et al. (2011) who demonstrated that milk production was as good as, or better, in diets where rapeseed meal replaced soybean meal. Although some adverse effects on reproduction efficiency in heifers have been reported following long-term feeding of high levels of rapeseed meal (Lindell, 1976; Lindell and Knutsson, 1976; Ahlström, 1978; Emanuelson et al., 1993; Ahlin et al., 1994), Emanuelson (1994) concluded that this was due to the presence of glucosinolates rather than erucic acid.

The seeds of *Crambe abyssinica* have the highest erucic acid content of all the oil seeds, and Böhme et al. (2005) used the meals from both crambe press cake (80.6 g erucic acid/kg DM) and solvent-extracted crambe seed meal (14.6 g erucic acid/kg DM) to examine the effect of erucic acid on

feed intake and milk production by lactating dairy cows. The cake and meal were included at 0%, 15% or 30% of the non-forage part of the ration DM and fed to 30 mid-lactation dairy cows (10 cows per treatment), resulting in erucic acid contents ranging from 0.1 to 24 g/kg DM for the crambe press cake ration and 0.1 to 3.9 g/kg DM for the crambe seed meal ration. Erucic acid intakes were 0.5, 46.2 and 108 g per day on the crambe press cake-supplemented diets and 0.6, 11.1 and 20.0 on the crambe seed meal diets. These intakes correspond to doses ranging from 0.00092 to 0.166 g erucic acid/kg bw, assuming a live weight of 650 kg. At 30% inclusion in the crambe press cake and crambe seed meal diets, intake of the non-forage feeds decreased by about 10%, but this was compensated for by an increase in forage intake. As a result, energy intake between groups was similar. At the highest level of inclusion on both the crambe press cake and crambe seed meal diets there was a reduction in milk yield, but this failed to reach statistical significance.

Hristov et al. (2011) fed high erucic acid low-glucosinolate rapeseed meal (42 g erucic acid/100 g total fatty acid) to eight multiparous Holstein lactating dairy cows. The erucic acid concentration in the diet was 9.6 g/kg DM. This concentration corresponds to a dose of 0.42 g/kg bw per day, based on a feed intake of 28.3 kg DM per day and a default live weight of 650 kg. Both DM intake and milk yield were significantly reduced by 8.4% and 4.4%, respectively ($p = 0.001$ and 0.047, respectively) compared to cows fed conventional solvent-extracted rapeseed meal (erucic acid not detected), although the feed efficiency for fat-corrected milk was increased by feeding the high erucic acid feed compared with the control. The inclusion of high-oil seed meals in the diet lowered rumen acetate concentration and the molar acetate:propionate ratio.

Based on the study of Böhme et al. (2005) it would appear that an erucic acid intake of 0.17 g/kg bw per day may have no adverse effect on milk yield, while intakes of 0.4 g erucic acid/kg bw per day may result in reductions in feed intake and milk yield (Hristov et al., 2011). However, the possible role of glucosinolates or other antinutritional factors in the meal could not be ruled out. The CONTAM Panel concluded that there were insufficient published data from which to derive a reliable NOAEL.

3.3.5.2. Pigs

Toxic effects in pigs

A description of the toxic effects caused by erucic acid in pigs is given in Section 3.3.2 and Appendix G, Table G.2. The CONTAM Panel identified a NOAEL of 0.7 g/kg bw per day from the study by Kramer et al. (1990) for increased myocardial lipidosis in newborn piglets exposed for 2 weeks.

Production-related effects in pigs

A number of studies have been undertaken in which production-related effects in pigs following dietary supplementation with rapeseed oil were reported.

Kramer et al. (1998) fed newborn piglets sows milk or one of four milk replacer diets supplemented with vegetable oils including HEAR. Erucic acid doses were 0, 0.02, 0.35, 0.39 and 3.5 g/kg bw per day (see Appendix G, Table G.2. for more information). After 4 weeks on experiment the weight gain of the pigs on the erucic acid free soybean oil treatment was significantly ($p < 0.05$) higher (6.8 ± 0.5 kg) than those on the HEAR oil diet (5.0 ± 0.4 kg), but no diet-related gross or histological abnormalities were observed among the piglets.

Friend et al. (1975) fed growing and fattening boars and gilts on diets supplemented with soybean oil or three rapeseed oils with varying levels (1.6%, 4.3% and 22.3%) of erucic acid. The oils were included at either 5% or 10% of the diet. See Appendix G, Table G.2 for further details. Although there was a small reduction in weight gain ($p < 0.05$) at the highest level of inclusion of the HEAR oil at the end of the first 4 weeks of the study, there were no effects on feed intake or live weight gain after 16 weeks. There were slightly higher feed intakes and live weight gains (non-significant) for the LEAR oil diets, such that the live weight gain and feed intakes resulted in a significantly ($p < 0.05$) improved feed conversion efficiency for the pigs on the LEAR oil diets. In a second study these authors fed boars diets supplemented with 20% soybean or HEAR oil (22.3% erucic acid). The pigs were on experiment for 16 weeks. Feed intake, average daily live weight gain and feed efficiency were significantly lower ($p < 0.01$) in pigs on the rapeseed oil-supplemented diet.

In a subsequent series of experiments, Friend et al. (1976) fed boars diets without supplementation (control) or supplemented with 25% of corn oil, LEAR or HEAR oils for 24 weeks. Erucic acid doses were 0, 0.1 and 1.6 g/kg bw per day (see Appendix G, Table G.2 for further details). There were no treatment effects on average daily gain; however, feed intakes on the oil-supplemented diets were significantly lower ($p < 0.05$) than those on the control diet. Feed intakes and weight gain of boars on

the rapeseed oil-supplemented diets were not significantly different to pigs on the corn oil, suggesting that the production effects observed were due to the total oil content of the diet rather than the presence of erucic acid.

Böhme et al. (2005) used crambe press cake and solvent-extracted crambe seed meal to examine the effect of erucic acid on feed intake and live weight gain of 100 crossbred pigs during their growing (26–60 kg bw) and fattening (60–120 kg bw) periods. Pigs on the control treatment (erucic acid content 0.2 g/kg DM) achieved daily live weight gains of 782 g/day. The inclusion of 5% and 10% crambe press cake gave diets containing 3.1 and 7.1 g erucic acid/kg DM in the growing period and 4.3 and 7.8 g erucic acid/kg DM. in the finishing period. The equivalent figures for crambe seed meal were 0.4 and 0.7 (5% and 10% inclusion, respectively) in the growing period and 0.3 and 0.7 g erucic acid/kg DM in the finishing period. Total intake of erucic acid ranged from 47 (control) to 1,912 g (crambe press cake, 20% inclusion). The pigs were fed a restricted intake for an average live weight gain of 750 g/day, and therefore, there were no differences between treatments in feed intake. Only the highest level of erucic acid (crambe seed meal 20%, 7.8 g erucic acid/kg DM) resulted in a 10% reduction in growth rate, to 742 g per day ($p < 0.05$). The inclusion of crambe press cake at 5% and 10% produced diets, containing 0.3 and 0.7 g erucic acid/kg f.w. resulted in small (1% and 3%, respectively) but not significant reductions in weight gain. There were also differences in the fatty acid composition of the body fat, but these did not result in significant changes in meat quality parameters such as juiciness, tenderness or taste. However, the CONTAM Panel have not used this study for the risk assessment of erucic acid due to the presence of glucosinolates in crambe press cake and solvent-extracted crambe seed meal to which pigs are particularly sensitive (Tripathi and Mishra, 2007; EFSA, 2008).

Aherne et al. (1975) compared HEAR (20.6%), LEAR (4.0%) and soybean oil in the diet for fattening pigs (25–90 kg bw) when included at a level of 15%. There were no differences in feed intake, live weight gain or feed conversion efficiency between pigs receiving HEAR (0.9 g/kg bw, see Appendix G, Table G.2 for further details) and those receiving LEAR (0.2 g/kg bw) or the soybean oil-supplemented diet.

In a subsequent study, Aherne et al. (1976) fed diets supplemented with HEAR oil (34.2% erucic acid in the fatty acid) or rapeseed oil from three low erucic acid cultivars (range 0.3–4.9% erucic acid in the fatty acid) to pigs from 19 to 130 kg bw. There were no significant differences between treatments in feed intake, average daily live weight gain or feed: live weight gain ratio. Exposure to erucic acid ranged from 0.02 to 2.6 g/kg bw per day (see Appendix G, Table G.2).

In summary, the effects of erucic acid on production-related parameters in pigs have mainly involved the use of oils from LEAR or HEAR cultivars, and in most studies the use of HEAR oil – which is not now used as a feed material - did not have an adverse effect on feed intake or growth rate. Although feed intakes by boars were significantly lower when fed diets containing rapeseed oil (no significant difference between diets supplemented with LEAR and HEAR oils, with erucic acid content 1 and 24 mol% of total fatty acids, respectively) compared to the control, but the authors (Friend et al., 1976) speculated that this was due to a higher consumption of the lower energy control diet to satisfy a common energy requirement. An earlier study by the same authors reported that exposure to 2.2 g erucic acid/kg bw per day resulted in a significant reduction in feed intake, average daily gain and feed efficiency (Friend et al., 1975). However, this contrasts with reports that exposures to 2.5 g erucic acid/kg bw per day had no adverse effect on feed intake or live weight gain (Aherne et al., 1976). Since none of the studies used erucic acid *per se*, the effects of other dietary constituents or characteristics, where production-related differences have been reported, cannot be ruled out.

3.3.5.3. Poultry

Toxic effects in poultry

Rations containing 25% of either HEAR oil (36% erucic acid), LEAR oil (1.9% erucic acid), soybean oil or a mixture of lard and corn oil were fed to chickens, ducks and turkeys for 52 days. Levels of erucic acid in the HEAR- and LEAR-supplemented diets were 90 and 4.75 g/kg, respectively. In the absence of default values for body weight and feed intake of young poultry species, the doses of erucic acid were not estimated. A significant decrease in body weight was observed in all species exposed to HEAR and the effect was most severe in ducks. HEAR oil exposure caused also anaemia. Relative liver weight was increased in all species fed HEAR oil. No ducks fed HEAR oil survived 28 days of age. Severe fatty change in the heart (myocardium), skeletal muscles, liver, spleen and kidney was found at an early age in all birds fed HEAR oil. Pale hearts and pale muscles were noted in all species

fed HEAR oil. Hydropericardium was observed in all chickens and ducks fed HEAR oil. A thickening of the epicardium and increased fibrous tissue in myocardium was noted in heart of ducks fed HEAR oil which died during the experiment. Some turkeys, fed HEAR oil had degenerative foci with infiltrations of histiocytic and giant cells in the myocardium (Ratanasethkul et al., 1976).

The effects of erucic acid on the reproductive performance of laying hens was studied by including 5% or 15% of high (*B. rapa*, 26.2% erucic acid) or low (*B. rapa*[45] cv. Span, 4.1% erucic acid) erucic acid rapeseed oils or soybean oil (0% erucic acid). These levels correspond to doses of 0, 0.1, 0.4, 0.8 and 2.4 g/kg bw per day using the default values of 2 kg body weight and a mean feed intake of 120 g/day (EFSA FEEDAP Panel, 2012). During a 28-day pre-treatment period all hens received a low-fat control diet. The treatment period lasting 56 days was divided into two periods of 28 days. Body weights of the laying hens in the different groups were not significantly different within the pre-treatment or within the treatment period. Feed consumption decreased significantly when the hens received diets containing 15% oil in the second treatment period. HEAR and LEAR oil-fed hens had the lowest and soybean oil-fed hens the highest daily feed intake when diets contained 5% oil. Per cent egg production was influenced significantly by the kind of oil in the diet. Significantly lower egg production was noted when hens were fed a diet containing 15% HEAR oil compared to hens receiving 15% soybean oil; the production being intermediate with LEAR oil (Vogtmann et al., 1974).

Single Comb White Leghorn cockerels (Hyline strain) were fed either an isocaloric basal (control) diet or a diet supplemented with 20% by weights of soybean oil or oil extracted from different rapeseed cultivars; one LEAR oil from *Brassica napus* cv. Tower (0.15% 22:1 n-9) and one LEAR oil from *B. rapa*[46] cv. Candle (1.3% 22:1 n-9), one HEAR oil extracted from a seed mixture of *B. rapa* cv. Echo[47] 85% and cv. Arlo 15% (30.66% 22:1 n-9) and one HEAR oil from *B. rapa* cv. R-500[48] (51.55% 22:1 n-9), a cultivar developed to produce oil for industrial purposes. Levels of erucic acid in the feed were: < 0.002%, < 0.002%, 0.03%, 0.3%, 6.1% and 10.3%, respectively. These levels correspond to doses of < 0.001, < 0.001, 0.02, 0.16, 3.7 and 6.2 g/kg bw per day using the default values of 2 kg body weight and a mean feed intake of 120 g/day (EFSA FEEDAP Panel, 2012). Three birds from each group were killed at 4, 8, 12 and 16 weeks. The mean body weight of birds fed control diet was significantly higher at week 8 and 12 than for birds fed the other diets. This difference was not evident at week 4 and had largely disappeared by week 16. Feeding 3.7 g/kg bw per day had no consistent effect on body weights, heart weights or heart-to-body weight ratio. Cardiac lipid levels consistently increased in the group receiving 6.2 g/kg bw per day. Significant *post-mortem* changes were reported in 12 birds from the group receiving 6.2 g/kg bw per day dying or been killed during the study. Two cockerels receiving 3.7 g/kg bw per day had similar gross lesions. Gross lesions were: a marked ascites resulting in distension of the abdominal wall, shrunken firm liver with uneven surfaces and thickened capsule, hydropericardium, dilation of the heart, thickened pericardium, oedema of the lung and marked muscle wasting, especially of the pectoral muscles. In addition, mineralisation of myocardium was noted in one bird and haemorrhage was found in the liver of two birds from the group receiving 3.7 g/kg bw per day. Histopathological changes were found in the brain, heart, liver and skeletal muscle. Healed encephalomalacia was observed in all groups. Heart lesions (vacuolation of myocytes indicating lipidosis, foci of degenerative and necrotic myocardium, fibrosis and in some birds mineralisation of the necrotic debris and formation of granulomas, scarring, mononuclear cell infiltration, signs of degenerate mitochondria) were observed in the two highest dose groups, the highest dose group being the most severely affected. Liver lesions (mild to moderate fatty metamorphosis, focal sinusoidal distension, periacinar necrosis, marked thickening of the capsule, increased fibrous connective tissue) were observed in all groups with an increase in the two highest dose groups. Mild bile duct hyperplasia was seen in some of these livers. Muscle lesions were also observed in all groups except controls with an increase in the two highest dose groups (hyaline degeneration of fibres of the superficial pectoral and semitendinosus, and cachetic muscular atrophy). Testes were examined for the presence of spermatids at 84 days but none were found. At 112 days, spermatids were found in a small number of birds in each treatment group. From this study, the CONTAM Panel identified a NOAEL of about 0.16 g/kg bw per day for heart lesions and a LOAEL of 0.02 g/kg bw per day for liver toxicity (increase incidence of sinusoidal distension) (Hulan et al., 1982; Corner et al., 1985).

[45] Reported by the authors as *B. campestris* cv. Span.
[46] Reported by the authors as *B. campestris* cv. Candle
[47] Reported by the authors as *B. campestris* cv. Echo
[48] Reported by the auhrors as *B. campestris* cv. R-500.

Male White Leghorn chicks (5/group) were fed basal diet or diets supplemented with soybean oil or four different rapeseed oils at 20% by weight of the diet from day 1 of age to 3 days, 1, 2 and 4 weeks. The four rapeseed oils contained 0.9%, 1.6%, 4.3% and 22.3% erucic acid.[49] Chicks fed diet containing rapeseed oils grew at a slower rate than chicks fed basal diet or diet containing soybean oil. Retardation in body weight was most pronounced in the HEAR group. After the first week, there was no significant difference in heart weights of the chicks in the different groups. The total cardiac lipid was consistently higher in chicks from the HEAR group throughout the feeding trial. Chicks from the other rapeseed oil groups also showed elevated levels of cardiac fat within the first week compared to chicks fed the basal diet, but these differences had disappeared by week 2 (Kramer and Hulan, 1977b).

Abdellatif and Vles (1970c) reported a series of experiments in Pekin ducklings. Firstly, 1 or 7-day-old Pekin ducklings were fed a diet containing various levels (10%, 20% and 30% by weight) of rapeseed oil with an erucic acid content of 40% for 20 days.[50] In addition to cardiac lipidosis, the animals showed severe hydropericardium and liver cirrhosis in the two highest dose groups. A mortality of 80% was noted in the highest dose group. In a dose–response study, ducklings were fed isocaloric diets containing 5%, 10%, 12.5%, 15%, 17.5% and 20% by weight of rapeseed oil (50% erucic acid; dose of erucic acid not reported) for 3 weeks. Mortality occurred only in the highest dose group, which also showed significant growth retardation in comparison with the other groups. Reticulocyte count and haematocrit increased with increasing level of rapeseed oil in the diet. There was a dose-related increase in heart weights (absolute and relative). Absolute mean liver weights increased with rapeseed oil levels up to 15% but decreased at higher doses. The following histopathological changes were observed at rapeseed oil levels $\geq$ 15%: hydropericardium, vacuolation, cell infiltration and oedema of myocardium and skeletal muscles, cirrhotic changes, centrolobular necrosis, engorgement of the liver and atrophy of the red pulp and increased erythropoiesis and lipid-laden cells in the spleen. In a third experiment, seven-day-old ducklings were fed an isocaloric diet containing 8.8% erucic acid (supplied by rapeseed oil (18.5% by weight) or glycerol trierucate (10% by weight) for 3 weeks. A control group received 20% hardened palm oil. The results indicate that the animals fed glycerol trierucate grow as poorly and develop the same lesions (hydropericardium, vacuolation of the myocardium and skeletal muscles and cirrhotic changes of the liver) as the animals fed rapeseed oil. However, the lesions were slightly severer in the rapeseed oil group which showed also mortality (3/10 ducklings) than those in the glycerol trierucate group which showed no mortality.

Abdellatif and Vles (1971a) investigated the effects of supplementing HEAR with olive oil, safflower oil or tallow in diets for 1-week-old Peking duck for 3 weeks. The diets contained (1) 30% by weight olive oil, (2) 30% by weight rapeseed oil (27.8% erucic acid in the diet), (3) 20% by weight rapeseed oil (18.5% erucic acid in the diet), (4) 20% by weight rapeseed oil plus 10% by weight tallow (18.6% erucic acid in the diet), (5) 20% by weight rapeseed oil plus 10% by weight safflower (18.5% erucic acid in the diet) or (6) 20% by weight rapeseed oil plus 10% by weight olive oil (18.5% erucic acid in the diet). The highest mortality (7/10) and growth retardation were observed in the group receiving the highest dose of erucic acid (2). The characteristic pathological effects of HEAR (hydropericardium, myocardial vacuolation, vacuolation of skeletal muscles, degenerative changes of the liver and lipidosis in the spleen) were observed in the groups fed rapeseed oil, being more severe in group (2). The incidence of liver and skeletal muscle changes was not appreciably different among the different groups. No lesion was observed in the group receiving olive oil (1).

In a second experiment, the effects of supplementing diets isocaloric in fats and in erucic acid for 3 weeks with increasing levels of hardened palm oil or glycerol trilaurate were investigated in ducklings. Erucic acid was supplied either as rapeseed oil or as glycerol trierucate. A group fed 20% by weight hardened palm oil was used as control. Mortality was highest in the groups fed rapeseed oil alone or rapeseed oil with glycerol trilaurate and was absent or very low in the other groups. Growth was significantly lower in the groups given erucic acid (groups fed rapeseed oil, glycerol trierucate or rapeseed oil with glycerol trilaurate compared to the control group and groups given erucic acid with palm oil supplement. No lesion was observed in the control group. Increasing levels of palm oil decreased the incidence and severity of liver cirrhosis, hydropericardium and splenic lipidosis. Vacuolar changes of the skeletal muscles were not improved, but those of the heart became more severe.

[49] In the absence of default values for body weight and feed intake of chicks, the doses of erucic acid for chicks were not estimated.

[50] In the absence of default values for body weight and feed intake of ducklings, the doses of erucic acid for ducklings were not estimated.

Supplementing the rapeseed oil diet with glycerol trilaurate increased the severity of the lesions. The authors concluded that the nutritional and pathological properties of rapeseed oil in ducklings are determined by the excess of erucic acid and the deficit of palmitic acid in the diet (Abdellatif and Vles, 1971a).

When diets containing 25% by weight rapeseed oil with 25% or 4.3% erucic acid were fed to ducklings, severe toxicity as described in the earlier study and high mortality was observed in the high erucic acid group after 2 weeks. The surviving animals developed cardiac fibrosis after 3 months. The ducks of the low erucic acid group showed no mortality and hydropericardium, but some of them developed vacuolar changes of the heart and skeletal muscles. These lesions had largely disappeared after 3 months (Abdellatif and Vles, 1973b).

Summary on toxic effects in poultry

Cardiac lipid levels increased in cockerels or chickens receiving diets containing erucic acid. This increase disappears at low doses after 2 weeks, but persists in animals fed higher doses. Cardiac lesions occurred in cockerels at doses of 3.7 g/kg bw per day and above with vacuolation and degeneration of myocardial cells, necrosis, fibrosis and mineralisation, formation of granulomas, thickening of the epicardium, scarring, mononuclear cell infiltration, signs of degenerate mitochondria and dilation. Hydropericardium was also observed in chickens fed HEAR oil. In the liver, fatty metamorphosis, focal sinusoidal distension, necrosis, fibrosis and marked thickening of the capsule were observed. In skeletal muscles, hyaline degeneration of fibres and muscular atrophy were noted. These effects were also reported in animals fed control or soybean oil, but the incidence and severity of lesions were significantly higher in cockerels fed rapeseed oil and especially in those fed high doses of erucic acid. The CONTAM Panel identified a LOAEL of 0.02 g/kg bw per day based on liver toxicity.

Growth retardation was reported in chickens fed diets containing rapeseed oil compared to chickens fed soybean oil, especially at high dose of erucic acid.

In turkeys fed HEAR oil significant decrease in body weight, increase in relative liver weight, severe fatty change (at an early age) in the heart, skeletal muscles, liver, spleen and kidney were observed. In addition, degenerative foci with hystiocytic infiltration and giant cells occurred in the myocardium.

Several studies in ducklings confirm the observations in other species: growth retardation, cardiac lipidosis, hydropericardium, myocardial vacuolation, vacuolation of skeletal muscles, liver cirrhosis resulting in mortality in highest dose erucic acid fed animals.

Production-related effects in poultry

In addition to the pathological effects described above, many studies have been undertaken to examine production-related effects of feeding full-fat rape seed, rapeseed meal and rapeseed oil on poultry. Many of the early studies showed that supplementation of diets with high erucic acid (HEAR) oils resulted in increased mortality and reductions in feed intake, live weight gain, egg production and egg weight (Abdellatif and Vles, 1971a, 1973b; Vogtmann et al., 1973; Clement and Renner, 1977). Abdellatif and Vles (1973b) attributed the increased mortality in ducks fed HEAR oil (50% erucic acid) to refusals by the birds to consume the feed, resulting in starvation.

Following the development of LEAR varieties, research has confirmed that full-fat rape seed, rapeseed meals and rapeseed oils can be used as feed for poultry. Early LEAR varieties included Tower and Candle, and Clandinin et al. (1978) found that both varieties could be included at 20% in diets for broilers without adverse effects, while increasing to 30% resulted in a small decrease in growth rate but no effect on feed conversion efficiency or mortality was observed. These authors also reported that Tower rapeseed meal could be included in diets of layers without adverse effects on feed intake, egg production or egg weight. Similar results were reported for Candle rapeseed meal (Slinger et al., 1978). For turkeys, Salmon (1979) reported that both Candle and Tower rapeseed meals could be successfully included at up to 30% in turkey diets. Based on an extensive review, Fenwick and Curtis (1980) concluded that rations for broilers and turkeys could be supplemented with up to 30% rapeseed meal, or 20% full-fat rape seed, without adverse effects on feed intake or growth rate. At that time, recommended levels for laying hens were lower because of the risk of producing eggs with a fish-flavour taint (Appendix D.2.2).

Sim et al. (1985) fed broiler chicks diets containing canbra oil (5.1% erucic acid) or sunflower oil which contained no erucic acid, with or without the addition of the free form of erucic acid, such that erucic acid contents of the diets were 0%, 40.5% and 42.0% of fatty acids. The feed intake and live weight of chicks at 28 days fed the canbra oil diet were significantly smaller than those fed the

sunflower oil diet, while the addition of erucic acid to the sunflower oil resulted in reduced feed intake and final live weight relative to the controls ($p < 0.05$) on the sunflower oil-containing diet.

In summary, early studies in which poultry were fed diets supplemented with oils and meals derived from HEAR cultivars clearly demonstrated adverse effects on production-related factors. However, as for other livestock, the possible effects of other dietary constituents or characteristics on feed intake, growth rate and egg production cannot be excluded. At least one study attributed the high oil content of the diet to the reluctance by the birds to consume the feed, resulting in starvation (Abdellatif and Vles, 1973b). Similarly, feed intakes were significantly reduced when diets were supplemented with 15% oil irrespective of the levels of erucic acid in the diet (Vogtmann et al., 1974). Consequently, the CONTAM Panel has been unable to derive any LOAEL or NOAEL for production-related characteristics for poultry.

3.3.5.4. Rabbits

One study on the toxic effects caused by erucic acid in rabbits was identified and is described in Appendix G, Table G.4. No NOAEL for rabbits could be identified.

3.3.5.5. Fish

The CONTAM Panel has been unable to identify studies that have specifically examined the adverse effects of erucic acid in fish. Numerous studies have examined the effect of including rapeseed meal and other rapeseed products on feed intake, growth rate, physiological and biochemical parameters of aquatic animals, but in most cases the levels of erucic acid in the diets used have not been given (e.g. Yurkowski et al., 1978; Higgs et al., 1982; Lanari and D'Agaro, 2005). In addition, the identified studies used fish oil or meal as a control. Since in these experimental settings, erucic acid is not the only source of variation in fatty acid composition between fish oil/meal and rapeseed oil/meal, these studies were not considered relevant for the risk assessment and the CONTAM Panel could not conclude on a NOAEL. Assuming that the erucic acid contents in the rapeseed products used in the studies do not exceed maximum permitted levels, then the inclusion of rapeseed meal at up to 30% of the diet is unlikely to result in adverse effects on growth or fish health (Lim et al., 1998; Burel et al., 2001; Enami, 2011). Similarly, rapeseed oil may replace fish oil without any adverse effects on growth rate or feed conversion efficiency.

3.3.5.6. Horses

Harris et al. (1999) fed eight thoroughbred horses diets that provided 1 g erucic acid/day (equivalent to approximately 0.002 g/kg bw per day) in vegetable oil for 10 months. No significant effects on glucose tolerance test, or on haematological parameters, monitored monthly, were observed. Total protein and gamma glutamyl transferase remained within the normal range throughout.

3.3.5.7. Companion animals

No studies on adverse effects in companion animals were identified.

3.3.6. Consideration of critical effects, dose response assessment and derivation of a health-based guidance value

3.3.6.1. Considerations of critical effects

The epidemiological studies were considered insufficient as a basis for risk assessment. The CONTAM Panel noted, however, that cardiac lipidosis shown in the animal studies, is relevant also for human health. The finding of an association between congestive heart failure and 22:1 fatty acids (Imamura et al., 2013), is consistent with animal studies, since associations between myocardial lipidosis (also termed steatosis in clinical studies) and heart failure (diastolic dysfunction) has been shown in humans (Lindsey and Marso, 2008; Rijzewijk et al., 2008; Wei et al., 2016). The therapeutic use of Lorenzo's oil (containing erucic acid) in ALD patients results in haematological effects, most notably thrombocytopenia and morphological alterations of thrombocytes, at erucic acid doses of about 0.1 g/kg bw per day.

A large body of evidence has been published, starting from the 1960s, showing that HEAR oils have toxic effects on the heart in experimental animals. Administration of dietary HEAR oils to rats, pigs and monkeys results in myocardial lipidosis i.e. an accumulation of triacylglycerols in myocardium that appear as neutral lipid droplets. The basis for this large accumulation of neutral lipid is likely to be the

poor β-oxidation of erucic acid by mitochondria. The lipid droplets are adjacent to, and in close contact with, the mitochondria. Mitochondrial damage has been reported only after exposure to HEAR oil for several months (e.g. degeneration of mitochondria in pigs). Prolonged administration of high erucic acid oils is associated with the development of multiple microscopic foci of myocardial necrosis, leading to myocyte loss and fibrosis, particularly common in the rat. Repeated exposure to HEAR oils has been associated with signs of cardiac distress (e.g. systolic and diastolic dysfunction in rats, degeneration of mitochondria in pigs) even if the levels of free fatty acids do not reach the levels required to significantly impact on the heart function.

However, the acute increases in neutral lipids within the myocardial cells are reversible, questioning whether the accumulation of these lipids might be a direct mediator of irreversible injury. Accordingly, administration of erucic acid during a few days produces a marked relative increase in neutral lipid content but there is no evidence that this may result in functional myocardium alteration or necrosis. Factors other than, or in addition to, erucic acid may be responsible for the increased incidence of these lesions.

Based on these considerations, the CONTAM Panel decided:

1) not to establish an acute reference dose because of the lack of endpoints indicative of acute toxicity on target organs;
2) to select myocardial lipidosis as critical effect for establishing a tolerable daily intake (TDI) for erucic acid.

The CONTAM Panel considered that the approach followed for the establishment of the TDI is conservative. Indeed, the effect selected, myocardial lipidosis, is transient and reversible: erucic acid induced lipidosis regresses after prolonged exposure and was reversible after return of the animals to a low-fat diet without erucic acid.

Upon repeated feeding with erucic acid-containing diets, non-cardiac effects such as changes in the liver, kidneys, skeletal muscle, adrenals and testis weight have also been reported in rats and haematological effects as well as liver morphological alterations in newborn piglets. In all cases, these effects are observed at somewhat higher doses (LOAELs of 4–13 g/kg bw per day) than those leading to cardiac lipidosis in the same species.

3.3.6.2. Dose–response assessment and derivation of a health based guidance value

Since there are no adequate data from human studies for dose–response assessment, the CONTAM Panel considered the data from studies on experimental animals to identify reference points.

Regarding myocardial lipidosis, six studies were identified in rats and one in pigs that were conducted in a wide dose-range and where erucic acid was reported as the main source of variation in fatty acid composition of the diet (see Section 3.3.2.2). No studies with these characteristics were identified in monkeys. Lipidosis is a complex endpoint characterised by several factors (i.e. incidence and severity) that should be taken into account for the identification of a reference point. Considering that incidence was generally high in control animals, that appropriate controls were not always available and that severity was not always reported quantitatively, the CONTAM Panel selected the NOAEL approach to identify the reference point. In rats, the NOAELs for myocardial lipidosis ranged from 0.7 to 2.6 g/kg bw per day and the LOAELs ranged from 1.0 to 7.1 g/kg bw per day (Table 11). In newborn piglets the NOAEL was 0.7 g/kg bw per day and the LOAEL was 1.1 g/kg bw per day (Table 12).

The CONTAM Panel selected the overall NOAEL for lipidosis of 0.7 g/kg bw per day, observed in a 7-day feeding study in young rats and in a 2-week feeding study in newborn piglets, as reference point for the risk assessment. Based on this NOAEL, the CONTAM Panel established a TDI of 7 mg/kg bw for erucic acid using the default uncertainty factor of 100 to account for intra- and interspecies differences. No additional uncertainty factor was applied to extrapolate from the short-term to long-term duration of the exposure because lipidosis is transient and reversible. The CONTAM Panel noted that this TDI is well below the erucic acid dose of 100 mg/kg bw causing haematological effects in ALD patients treated with Lorenzo's oil.

3.4. Risk characterisation

3.4.1. Human health risk characterisation

The CONTAM Panel established a TDI of 7 mg/kg bw for erucic acid based on a NOAEL for myocardial lipidosis in rats and pigs.

Data on human dietary exposure levels of erucic acid across dietary surveys and age groups are presented in Table 6 and show mean exposure values that range from 0.3 (minimum LB) to 4.4 mg/kg bw per day (maximum UB), across dietary surveys and age groups. Mean dietary exposures for adults ranged from 0.3 to 1.9 mg/kg bw per day across European surveys and was highest for toddlers, ranging from 1.2 to 4.4 mg/kg bw per day. These exposures are below the TDI of 7 mg/kg bw per day.

The 95th percentile dietary exposure levels range from 0.7 (minimum LB) to 9.5 mg/kg bw per day (maximum UB) across dietary surveys and age groups, with a range for adults from 0.9 to 4.3 mg/kg bw per day. The 95th percentile dietary exposure level was highest in infants (ranging from 1.7 to 7.4 mg/kg bw per day, minimum LB – maximum UB) and 'Other children' (ranging from 2.1 to 9.5 mg/kg bw per day, minimum LB – maximum UB), the last maximum UB estimate being above the TDI. This may indicate a risk for young individuals with high erucic acid exposure. However, it should be noted that only one exposure estimate (UB) was above the TDI across all the different dietary surveys.

3.4.2. Animal health risk characterisation

Dietary exposure to erucic acid from rapeseed meal and oil was calculated for different livestock species (Tables 8 and 9). In the absence of any feed industry data on levels of rapeseed meal and oil in livestock feeds in the EU, the CONTAM Panel has used maximum recommended inclusion levels from industry guidelines (Ewing, 1997; Canola Council of Canada, 2015). Therefore, levels of exposure calculated in this opinion represent worst-case scenarios. It should be mentioned that insufficient data on levels of erucic acid in rapeseed meal or cake were available to allow P95 estimates of exposure to be made. The CONTAM Panel identified a NOAEL of 700 mg/kg bw per day for myocardial lipidosis in newborn piglets. Production-related effects were only observed at doses higher than the NOAEL for lipidosis. The dietary exposure of pigs ranges from 3.9 (minimum LB) to 8.6 mg/kg bw per day (maximum UB), which is well below the NOAEL for lipidosis in pigs.

For poultry, a LOAEL of 20 mg/kg bw per day was identified for liver toxicity, which is about double the upper exposure range (4.4 (minimum LB) to 12 mg/kg bw per day (maximum UB)). The small margin between the LOAEL and the estimated exposure may indicate a health risk for poultry where maximum inclusion rates are applied.

The available database on adverse effects in ruminants was too limited to identify an overall NOAEL. However, no effect on milk yield was observed in the study by Böhme et al. (2005) at a dose of 170 mg/kg bw per day. The dietary exposure of dairy cattle ranges from 1.8 (LB) to 2.6 mg/kg bw per day (UB), which is well below the dose at which no effect is observed on the milk yield. However, the risk of other adverse effects or for other ruminants could not be assessed.

No NOAEL/LOAEL could be identified for horses, fish and rabbits, and therefore, the risk of erucic acid exposure could not be assessed for these species. However, the CONTAM Panel noted that the exposure of horses (0.95/1.5 mg/kg bw per day (LB/UB)) and rabbits (6.4/13 mg/kg bw per day (LB/UB)) is well below the NOAEL of 700 mg/kg bw per day for pigs.

Since rapeseed meal and rapeseed oil are not commonly used in the diets of cats and dogs, the dietary exposure to erucic acid is considered to be negligible and no risk characterisation has been undertaken.

3.5. Uncertainty analysis

3.5.1. Assessment objectives

The objectives of the assessment were clearly specified in the terms of reference.

3.5.2. Exposure scenario/exposure model

A final dataset of 12,444 food samples was available to estimate chronic dietary exposure to erucic acid. Apart from the samples of rapeseed oil that were mainly reported as collected in the European Union without further details, most of the other food samples (~ 75%) were collected in one Member State. Therefore, there is uncertainty on whether possible country-based differences in the levels of erucic acid in diverse food commodities are well represented; this may affect for instance food commodities such as 'Fine bakery wares' particularly important in the dietary exposure to erucic acid. Likewise, the lack of information on the analytical method used to analyse some food samples (~ 30%) adds some uncertainty to the levels of erucic acid reported for some food commodities. There

is also uncertainty in the occurrence values of few food samples for which the fat content was imputed, as this information was originally not provided.

In relation to exposure assessment, uncertainties and limitations related to the use of the EFSA Comprehensive Food Consumption Database have already been described in EFSA (EFSA, 2011a) and are not further detailed. Among those with a particular implication for the dietary exposure to erucic acid, the uncertainty associated to the eating occasions reported for 'Composite food' should be mentioned as no information is available on whether they referred to homemade dishes or ready-to-eat foods. This is particularly important in the estimation of dietary exposure to erucic acid, and, together with the different level of disaggregation reported in the consumption data and the low number of occurrence data reported, made it necessary to carry out specific exposure scenarios for these food commodities. The specific exposure scenarios for 'Custard' and 'Chutneys and pickles' are surrounded by uncertainty due the low number of samples reported. In particular, the exposure scenario for the latter group is surrounded by further uncertainty as the low number of samples raises doubts about the representativity of a rather heterogeneous food group, and the fact that the number of eating occasions in the Comprehensive database for 'Chutneys and pickles' is very low.

There is also uncertainty associated to the contribution of 'Fish and other seafood' since in both the Comprehensive and the occurrence database for certain reported data there is no clear information on whether they refer to oil canned samples (and the type of oil used) or not. In addition, misidentification between erucic acid (22:1 n-9) and cetoleic acid (22:1 n-11) in fish and seafood is relatively common during fatty acid analysis. Consequently there is uncertainty regarding the reported levels of erucic acid in fish and other seafood in the occurrence data reported to EFSA as well as in food nutrients databases and scientific literature. Misidentification between erucic acid and other 22:1 fatty acids may also occur in other foods as for example due to the presence of *trans* and positional isomers after partial oil hydrogenation. Therefore, there is some uncertainty associated to the occurrence data and subsequently to the exposure estimations.

Further uncertainty is identified when excluding different types of vegetable oils (olive oil, sunflower oil, among others) that do not naturally contain erucic acid but for which levels of this fatty acid were reported. The presence of such adulterated oils in the market would imply an increase in the dietary exposure to erucic acid. This increase would be rather small in most of the dietary surveys under the LB scenario, and would imply a maximum of 1.9-fold/1.6-fold increase under the UB scenario (mean/95th percentile dietary exposure). Since the presence of erucic acid was reported for very few samples of these vegetable oils, exposure estimations under the UB would be surrounded by large uncertainty. In any case, the influence of these samples is negligible for the maximum estimates of the mean and 95th percentile dietary exposure across the different dietary surveys.

Dietary exposure to erucic acid may be underestimated to a certain extent due to the lack of occurrence data in animal-derived foods and the evidence that erucic acid in the feed is transferred to products of animal origin.

The animal risk assessment is hampered by limited representative feed consumption data for livestock and fish across Europe. Furthermore, insufficient data on levels of erucic acid in rapeseed meal or cake were available to allow P95 estimates of exposure to be made. As a result, there is considerable uncertainty regarding the total dietary exposure to erucic acid in the animal risk assessments. Due to the limited data on the amount of rapeseed meal/oil used in animal diets, the CONTAM Panel has used published maximum recommended inclusion rates for each of the livestock categories which is likely to have overestimated exposure in most cases, although the extent of this is uncertain.

Rapeseed meal is a major feed ingredient and likely to be the single most important source of erucic acid for all livestock. While in excess of 6 million tonnes of rapeseed meal are used as feed for livestock in the EU, data for only 28 samples were reported. Furthermore, these were classified as rapeseed *expeller*, while the *meal* – which would be expected to have a lower erucic acid content - is the more commonly used feed material. The calculation of exposure was therefore based on a very limited number of quantified results, which has added to the uncertainties of exposure estimates.

3.5.3. Model input (parameters)

Performance criteria for the analytical methods used for the official control of levels of erucic acid in foodstuffs are laid down in Commission Regulation (EC) No 2015/705 and no specific methods are prescribed at EU level.

The analytical results used for the exposure assessment were performed by different laboratories at largely varying LOQ/LODs. This fact together with the large proportion of samples with left-censored

data (69%) introduces uncertainty to the overall dietary exposure estimates. While the LB values tend to underestimate the chronic dietary exposure to erucic acid, UB values tend to overestimate it.

3.5.4. Other uncertainties

Present knowledge on the absorption, distribution and excretion of erucic acid in animal models and humans is based on old studies using high doses of rapeseed oil with high levels of erucic acid and employing imprecise methods. These data do not necessarily reflect the toxicokinetic situation when low levels of glycerol erucates are present in the diet.

Although the metabolic fate of erucic acid in the mammalian organism is well known in qualitative terms, there is only preliminary information about quantitative metabolic differences between humans and various animal species used for toxicity testing. The metabolic degradation of erucic acid in cardiac and hepatic mitochondria and peroxisomes appears to play a major role in the mode of action and may explain observed species differences in the toxicity of erucic acid, but data on the activity and inducibility of these organelles are scant. Another uncertainty relates to the implication of physiological differences (rate and strength of contraction, and energy demand) of the heart between species for the toxicological sequelae of erucic acid.

Many toxicological studies are decades old, do not meet current standards and were not designed to determine a reference point. No pure erucic acid was administered to the experimental animals, but they were fed diets containing oils with various levels of erucic acid, mainly rapeseed oil but also other vegetable oils. These vegetables oils contain besides erucic acid, other fatty acids and other nutrients, which may have harmful or protective effects. Thus the dietary background may have a large influence on the outcome of the studies. In most toxicological studies, oil enriched feed was used which is particularly energy dense. Since most studies did not report the used doses, the CONTAM Panel used default values to convert levels in the diet into doses. However, due to the high calorie content of oil enriched feed, experimental animals do not eat such feed as much as they would eat standard feed and the actual doses may be lower than the calculated doses. This may result in an overestimation of the NOAEL. In addition, the use of oil enriched feed may lead to compromised levels of some micronutrients. According to a study in which the feed was enriched with 20% of fish oil, the rats in the control groups had approximately 30% lower feed intake compared to rats fed standard laboratory rodent feed (Vaskonen et al., 1996). Some studies did not report the weight % of erucic acid in the oil but the calorie %. In this case, the CONTAM Panel assumed that the weight % equals half of the calorie %, an assumption that is used in many studies. Several studies reported only the level of 22:1 or 22:1 n-9 (without specification of *cis* or *trans* configuration) in the oil. Since erucic acid is by far the most important 22:1 fatty acid in rapeseed oil, the CONTAM Panel assumed that all 22:1 present in rapeseed oil is erucic acid. Overall the use of studies with rapeseed oil and the assumptions for dose calculations adds to the uncertainty.

The critical effect selected for the derivation of the health-based guidance value (HBGV) is myocardial lipidosis in experimental animals. This is regarded as a short-term, reversible effect, and therefore, it is not a direct mediator of irreversible injury. However, when myocardial lipidosis is observed following feeding with HEAR oils, myocardial distress is also described. Moreover, myocardial lipidosis has never been reported in humans exposed to erucic acid and an extrapolation from effects on the rat or pig heart to the human heart is complicated by differences in physiology.

Other antinutritive factors present in rapeseed meal may also have contributed to any adverse effects reported in livestock. Regarding production-related adverse effects in livestock, relatively few studies have reported all the information necessary to estimate exposure (feed intake and live weights of the species, erucic acid contents of the feed), thus adding further uncertainty to the levels of exposure at which adverse effects may be observed. In addition, many studies were done 40 years or more ago, and therefore, their applicability for the current genotypes is uncertain.

3.5.5. Summary of uncertainties

In Table 13, a summary of the uncertainty evaluation is presented, highlighting the main sources of uncertainty and indicating an estimate of whether the respective source of uncertainty might have led to an over- or underestimation of the exposure or the resulting risk.

Table 13: Summary of qualitative evaluation of the impact of uncertainties on the risk assessment of erucic acid in food and feed

Sources of uncertainty	Direction
Use of LB and UB occurrence data in the exposure estimations	+/–[(a)]
Exclusion of composite foods from the general scenario	–
Lack of occurrence data on foods of animal origin	–
Misidentification of erucic acid during the analytical measurements	+/–
Exclusion of different types of vegetable oils for human dietary exposure	–
Imputation of fat content in certain foods	+/–
Extrapolation of occurrence data to the whole of Europe	+/–
Use of maximum inclusion levels of rapeseed meal and oil in livestock diet	+
Few occurrence data in feed	+/–
Insufficient data on levels of erucic acid in rapeseed meal or cake to allow P95 estimates of livestock dietary exposure	–
Lack of quantitative studies on the absorption, distribution and excretion of erucic acid from dietary glycerol erucates	+/–
Lack of comparative studies on the activity of mitochondria and peroxisomes of humans, pigs, rats, and other animal species for the metabolism of erucic acid	+/–
Implication of physiological differences of the heart between species	+/–
Dose calculation of erucic acid in experimental animals	–
Influence of other fatty acids and other minor components in the oil, and of high-fat diet on the induction of lipidosis/necrosis in experimental animals	+/–
Limited studies on current genotypes of livestock	+/–
Dose calculation of erucic acid in livestock	+/–
Use of lipidosis as critical effect for establishing the HBGV	+

(a): + = uncertainty with potential to cause overestimation of exposure/risk; – = uncertainty with potential to cause underestimation of exposure/risk.

The CONTAM Panel considered that the impact of the uncertainties on the risk assessment of human and animal exposure to erucic acid through consumption of food and feed is considerable. Based on the lipidosis selected as the critical effect to derive the TDI, the CONTAM Panel concluded that the risk assessment of human exposure to erucic acid presented in the opinion is more likely to overestimate than to underestimate the risk. For the same reason, the CONTAM Panel concluded that the risk assessment for pigs is more likely to overestimate than to underestimate the risk. The use of maximum inclusion rates resulted in an overestimation of the exposure for poultry. However, considering the uncertainties related to the use of a LOAEL as toxicological reference point for poultry, it is not possible to conclude whether the risk for this species is overestimated.

4. Conclusions

Erucic acid is the trivial name of the fatty acid Z-13-docosenoic acid, abbreviated as 22:1Δ13c, although it is more frequently found in the literature as 22:1 n-9. Erucic acid is mainly present in the seeds of species of the Brassicaceae, which includes important seed crops such as rapeseed and mustards, and also important vegetable crops such as the diverse group of kales, cabbages and turnips. Cultivars of Brassicaceae with very low erucic acid content have been developed for seed oil production for food and feed use in most countries, including the EU. Mustard seed production is based on cultivars with high erucic acid content. Erucic acid is also present at low concentrations in other food sources such as fish. Erucic acid is present in Lorenzo's oil, a drug used for therapy for ALD patients. Erucic acid doses range from 0.09 to 0.51 g/kg bw per day.

4.1. Occurrence/exposure

- The dietary exposure was estimated using a final dataset of 12,444 food samples representing most of the food commodities with potential presence of erucic acid.
- Samples were collected between 2000 and 2015 (half of them in 2014) in 15 different European countries, but most of them from one Member State.

- The percentage of left-censored data reported (results below limit of detection and/or limit of quantification) was high (69%). The highest number of reported samples corresponded to the food group 'Animal and vegetable fats and oils' (~ 60%) and in particular to 'Rapeseed oil' (n = 5,832). Other food groups that were well represented were 'Starchy roots and tubers' (n = 1,223), 'Grains and grain-based products' (n = 982) and 'Food for infants and small children' (n = 810).
- Mean values reported in rapeseed oil were 1,285/5,215 mg/kg (LB/UB) with about 80% being left-censored data.
- The presence of erucic acid in 'Fine bakery wares' indicates the common use of rapeseed oil in the preparation of these products. For 'Pastries and cakes', erucic acid was quantified in half of the samples (mean 240/290 mg/kg (LB/UB)), and for 'Biscuits', in 25% of the samples (mean 270/390 mg/kg (LB/UB)).
- Within the food group 'Food for infants and small children', the highest mean values were reported for 'Infant formulae, powder' (220/290 mg/kg (LB/UB) and the lowest for 'Ready-to-eat meal for infants and young children' (77/86 mg/kg, (LB/UB)).
- Only 275 feed samples were available to estimate animal dietary exposure to erucic acid, most of the samples were collected in the European Union between 2003 and 2015.
- Most of the feed samples referred to 'Rapeseed oil' (n = 193). The erucic acid content of only 28 samples of rapeseed expeller was provided, and no data for rapeseed meal, which is the more commonly used feed. The highest average levels of erucic acid were reported for 'Rapeseed oil' (1,300/4,200 mg/kg (LB/UB)).
- The highest human chronic dietary exposure was estimated in the youngest population. For the mean dietary exposure, the highest estimate at the LB corresponded to the age classes 'Infants' and 'Toddlers' with a maximum value of 2.8 mg/kg bw per day, while at the UB the maximum estimate was observed in the age class 'Toddlers' (4.4 mg/kg bw per day). In the highly exposed population (95th percentile), the highest estimates were in 'Infants' (5.8/7.4 mg/kg bw per day (LB/UB)) and 'Other Children' (5.3/9.5 mg/kg bw per day (LB/UB)).
- Overall, the food group 'Fine bakery wares', more precisely 'Pastries and cakes' and 'Biscuits (cookies)' was the main contributor of dietary exposure to erucic acid. At the middle bound (MB), the contribution of 'Fine bakery wares' in 'Toddlers' represented up to 39% of the total exposure (median = 21%) and in 'Other children' contributed up to 48% to the total exposure (median = 27%).
- The contribution of rapeseed oil to the total dietary exposure to erucic acid was, in most of the cases, limited. However, in few dietary surveys, the consumption of rapeseed oil played an important role reaching average contributions (MB scenario) up to 63%, with an average contribution of 39% in the dietary survey with the highest exposure estimate.
- Since the levels of erucic acid in 'Fine bakery wares' were not that high (~ 200/400 mg/kg (LB/UB)), its relevant contribution is mainly driven by the high consumption of this heterogeneous food category (e.g. croissants, doughnuts, cakes, muffins, waffles, biscuits, cookies, etc.).
- In the age class 'Infants', 'Food for infants and small children' (FoodEx level 1) was the main contributor to the exposure. The contribution of the food group 'Ready-to-eat meal for infants and young children' reached 52% at the MB scenario (range 19–52%) among the dietary surveys for 'Infants'.
- Specific exposure scenarios (consumers only) were used to estimate the potential exposure via the consumption of 'Composite foods' and 'Custard'. Both maximum mean exposure (UB) and maximum 95th percentile dietary exposure (UB) via the consumption of prepared pasta alone were around sixfold higher than the maximum exposure estimates in 'Toddlers' and 'Adults' considering the whole diet.
- Exposures to erucic acid by farmed livestock were estimated using typical feed intakes and body weights, and feed industry guidelines for the maximum inclusion rates of rapeseed meal and oil in livestock diets. Therefore, mean levels of exposure calculated in this opinion represent worst-case scenarios. However, insufficient data on levels of erucic acid in rapeseed meal or cake were available to allow P95 estimates of exposure to be made. In the category 'Ruminants and horses', the highest exposure was for lactating goats (5.0/7.5 mg/kg bw per day (LB/UB)).
- For pigs and poultry, the highest exposure was for fattening chickens (9.4/12 mg/kg bw per day (LB/UB)).
- Since rapeseed oil or meals are not commonly included in diets for cats and dogs, no estimates of exposure have been made for these animals.

4.2. Hazard identification and characterisation

4.2.1. Toxicokinetics

- Erucic acid is well absorbed from the gastrointestinal tract to an extent varying between 60% and 100%, depending on the species.
- Erucic acid is distributed to all organs; however, there is little distribution into the brain.
- Mitochondrial β-oxidation of erucic acid is poor in rats and pigs. Human heart mitochondria appear to also have low activity for erucic acid.
- Little is known regarding the excretion of erucic acid.
- There is evidence that erucic acid in the feed is transferred to products of animal origin and a dose-related increase in erucic acid in food of animal origin has been shown.
- In ruminants, erucic acid is also partially hydrogenated or isomerised in the rumen.

4.2.2. Toxicity in experimental animals

- The heart is the principal target organ for toxic effects following short-term or long-term exposure of rats, pigs, monkeys, rabbits and gerbils to diets with oils containing erucic acid.
- The most common and sensitive effect observed in all species is myocardial lipidosis. This effect is reversible and transient during prolonged exposure. Myocardial lipidosis has been reported to reduce the contractile force of the heart muscle. The manifestation of cardiac lipidosis varies among species.
- The overall NOAEL for lipidosis was 0.7 g/kg bw per day in a 7-day feeding study in young rats and in a 2-week feeding study in newborn piglets.
- Feeding rats with high erucic acid doses for 4 or more weeks is associated with the occurrence of myocardial necrosis and fibrosis. Factors other than, or in addition to, erucic acid are likely to be responsible for the increased incidence of these lesions, e.g. fatty acid imbalance. Therefore, necrosis was not considered a suitable endpoint for the risk assessment. A causal link between myocardial lipidosis and myocardial lesions has not been established.
- Because of the lack of adequate studies, no conclusions can be drawn on the genotoxicity and carcinogenicity of erucic acid.
- No major adverse reproductive and developmental effects were associated with feeding female rats, mice and hamsters with erucic acid-containing diets prior to mating and during pregnancy.

4.2.3. Observations in humans

- A higher level of 22:1 in plasma phospholipids has been associated with higher incidence of congestive heart failure in two independent cohorts whereas higher circulating levels of erucic acid in erythrocytes have been associated with lower incidence of coronary heart disease.
- Two studies on the possible association between cancer and erucic acid exposure were identified but no conclusion can be drawn due to the intrinsic limitations or lack of specificity of the outcome.
- The therapeutic use of erucic acid results in haematological effects, most notably thrombocytopenia and morphological alterations of thrombocytes, at doses of about 0.1 g/kg bw per day.
- Erucic acid induced lipidosis has not been described in humans.

4.2.4. Mode of action

- A high intake of erucic acid leads to lipidosis in pigs and rats, particularly in the heart, due to the poor β-oxidation of erucic acid in mitochondria.
- Cardiac lipidosis is transient (reversible), even after prolonged intake of erucic acid because of the induction of peroxisomal degradation of erucic acid.

4.2.5. Adverse effect in livestock

- A reduction in feed intake and milk yield by dairy cows was reported at an intake of 0.4 g erucic acid/kg bw per day from rapeseed meal. However, the possible role of glucosinolates or other antinutritional factors in the meal could not be ruled out.

- For pigs, the CONTAM Panel identified a NOAEL of 700 mg/kg bw per day for myocardial lipidosis.
- Feeding poultry with diets containing HEAR oil resulted in growth retardation and cardiac lipidosis. High doses of erucic acid also increased the incidence and severity of cardiac lesions (similar to those observed in the rat). In addition, hydropericardium, effects on the liver and skeletal muscles were induced in several species fed diets containing high doses of erucic acid. The CONTAM Panel identified a LOAEL of 0.02 g/kg bw per day for liver toxicity in poultry.
- Studies in which poultry were fed diets supplemented with oils and meals derived from HEAR cultivars clearly demonstrated adverse effects on production-related factors. However, as for other livestock, the possible effects of other dietary constituents or characteristics on feed intake, growth rate and egg production cannot be excluded.
- No conclusion regarding the adverse effects in fish, rabbits and horses could be drawn due to the limited studies available.
- No studies on adverse effects in companion animals were identified.

4.2.6. Considerations of critical effects, dose–response modelling and possibilities for derivation of a health-based guidance value

- An acute reference dose was not established because of the lack of endpoints indicative of acute toxicity on target organs.
- Myocardial lipidosis, as reported in rats and pigs following feeding with HEAR oils, was selected as critical effect for establishing a tolerable daily intake (TDI) for erucic acid.
- Non-cardiac effects, such as changes in the liver, kidneys, skeletal muscle, adrenals and testis weight, have also been reported in rats and haematological and liver alterations in newborn piglets. In all cases, these effects are observed at somewhat higher doses than those leading to cardiac lipidosis in the same species.
- Since there are no adequate data from human studies for dose–response assessment, the CONTAM Panel considered the data from studies on experimental animals to identify reference points.
- The CONTAM Panel selected the overall NOAEL for lipidosis of 0.7 g/kg bw per day, observed in a 7-day feeding study in young rats and in a 2-week feeding study in newborn piglets, as reference point for the risk assessment.
- Based on this NOAEL, the CONTAM Panel established a TDI of 7 mg/kg bw for erucic acid using the default uncertainty factor of 100 to account for intra- and interspecies differences.
- The CONTAM Panel noted that this TDI is well below the erucic acid dose of 100 mg/kg bw per day causing haematological effects in ALD patients treated with Lorenzo's oil.

4.3. Risk characterisation

4.3.1. Human health risk characterisation

- Mean human dietary exposure to erucic acid across dietary surveys and age groups ranges from 0.3 (minimum LB) to 4.4 mg/kg bw per day (maximum UB) across dietary surveys and age groups, which is below the TDI of 7 mg/kg bw per day.
- The 95th percentile dietary exposure level was highest in infants (ranging from 1.7 to 7.4 mg/kg bw per day, minimum LB – maximum UB) and 'Other children' (ranging from 2.1 to 9.5 mg/kg bw per day, minimum LB – maximum UB), the last maximum UB estimate being above the TDI. This may indicate a risk for young individuals with high erucic acid exposure. However, it should be noted that only one exposure estimate (UB) was above the TDI across all the different dietary surveys.

4.3.2. Animal health risk characterisation

- For ruminants, no NOAEL could be identified. The dietary exposure of dairy cattle is well below the dose at which no effect is observed on milk yield. However, the risk of other adverse effects or for other ruminants could not be assessed.
- The dietary exposure of pigs is well below the NOAEL for myocardial lipidosis of 700 mg/kg bw per day.
- For poultry, a LOAEL of 20 mg/kg bw per day was identified for liver toxicity, which is about double the upper exposure range (12 mg/kg bw per day). The small margin between the

LOAEL and the estimated exposure may indicate a health risk for poultry where maximum inclusion rates are applied.
- No NOAEL/LOAEL could be identified for horses, fish and rabbits, and therefore, the risk of erucic acid exposure could not be assessed for these species. However, the CONTAM Panel noted that the exposure of horses (0.95/1.5 mg/kg bw per day (LB/UB)) and rabbits (6.4/13 mg/kg bw per day (LB/UB)) is well below the NOAEL of 700 mg/kg bw per day for pigs.
- The dietary exposure of cats and dogs to erucic acid is considered to be negligible and no risk characterisation has been undertaken.

5. Recommendations

- To generate more analytical data on the occurrence of erucic acid in relevant food and feed commodities using sensitive and specific methods. Special attention should be paid to processed foods such as 'Fine bakery wares', 'Food for infants and small children' and 'Composite foods'.
- There should be more information on the levels in animal-derived products (meat, milk and eggs) resulting from the transfer of erucic acid from animal feed.
- There is a need for a repeated-dose toxicity study in newborn rats or pigs with pure erucic acid in order to clarify the potential confounding effects of other fatty acids present in the oil and to provide information regarding the dose–response relationship.
- Studies should be conducted on species differences in the cardiac and hepatic metabolism of erucic acid.
- Further studies are required to determine reference points for target livestock animals and fish.

Documentation provided to EFSA

1) Henkel KGaA 1981a. Unpublished data, Archive-No. Dr. Kastner 945.
2) Henkel KGaA 1981b. Unpublished data, Archive-No. Dr. Wallat 265.
3) European Commission - European Chemicals Bureau 2000. IUCLID Dataset Substance ID 112-86-7. 94 p.

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Abbreviations

alb	albumin
ALD	adrenoleukodystrophy
AOAC	Association of Official Analytical Chemists
AOCS	American Oil Chemists' Society
AP	alkaline phosphatase
ARIC	Atherosclerosis Risk in Communities Study
ATP	adenosine triphosphate
ATR-FTIR	Attenuated total reflection-Fourier transform infrared
BIPEA	International Bureau for Analytical Studies
bw	body weight
CAD	coronary artery disease
Car	carnitine
CAS	Chemical Abstracts Service
CHD	coronary heart disease
CHF	congestive heart failure
CHS	Cardiovascular Health Study
Chylo	chylomicron
CoA	coenzyme A
CONTAM	EFSA Panel on Contaminants in the Food Chain
DATA	EFSA Evidence Management Unit
DHA	docosahexaenoic acid

DM	dry matter
EA	erucic acid
EC	European Commission
ECG	electrocardiogram
EFSA	European Food Safety Authority
ELOV1	elongation of very long-chain fatty acid
EPA	eicosapentaenoic acid
exp	experiment
f.w.	fresh weight
$FADH_2$	flavin adenine dinucleotide
FAME	fatty acid methyl esters
FEDIAF	European Pet Food Industry Federation
FEDIOL	European Vegetable Oil and Proteinmeal Industry
FEFAC	European Feed Manufacturers' Federation
FERA	Food and Environment Research Agency
FID	flame ionisation detectors
FSA	Food Standards Agency
FSANZ	Food Standards Australia and New Zeeland
FT-NIR	Fourier-transform near-infrared
GC	gas chromatography
GLA	γ-linolenic acid
GTE	glycerol trierucate
H_2O_2	hydrogen peroxide
Hb	haemoglobin
HBGV	health-based guidance value
HDL	High density lipoprotein
HEAR	high erucic acid rapeseed
HPLC	high-performance liquid chromatography
i.p.	intraperitoneal
IPCS	International Programme on Chemical Safety
IUPAC	International Union of Pure and Applied Chemistry
LB	lower bound
LC	left-censored
LD_{50}	lethal dose, median
LCMUFA	long-chain monounsaturated fatty acids
LCO	lard and corn oil
LEAR	low erucic acid rapeseed
LOAEL	lowest observed adverse effect level
LOD	limit of detection
LOQ	limit of quantification
LPL	lipoprotein lipase
Max	maximum
MB	middle bound
MDA	malonaldehyde
Min	minimum
ML	maximum level
MS	mass spectrometry
MUFA	monounsaturated fatty acids
N	Number
n.r.	Not reported
NADH	nicotinamide adenine dinucleotide
NHANES	National Health and Nutrition Examination Survey
NIR	near-infrared
NIST	National Institute of Standards and Technology
NMR	nuclear magnetic resonance
NOAEL	no observed adverse effect level
NOEL	no observed effect level

OECD	Organization for Economic Co-operation and Development
P/O	phosphate oxygen ratio
PCV	packed cell volume
PHFO	partially hydrogenated fish oil
PHHO	partially hydrogenated herring oil
PL	phospholipid
PPAR	proliferator-activated receptor
pTDI	provisional tolerable daily intake
PUFA	polyunsaturated fatty acids
RASFF	Rapid Alert System for Food and Feed
RBC	red blood cell
RES	reticuloendothelial system
RFO	refined fish oil
RSO	rapeseed oil
SBO	soybean oil
SCF	Scientific Committee on Food
SGOT	serum glutamic oxaloacetic transaminase
SNE	Specialised Nutrition Europe
SOP	standard operational procedure
SUN	sunflower seed oil
TDI	tolerable daily intake
TG	triacylglycerol
UB	upper bound
VLCFA	very long-chain fatty acid
VLDL	very low-density lipoproteins
WG	Working group
WHO	World Health Organization

Appendix A – Erucic acid content in rapeseed cultivars

Table A.1: Erucic acid content (% total fatty acids) in rapeseed cultivars marketed in France, from plants grown in multilocation experiments conducted in three different geographical areas of the country from 2005 to 2015 (Terres Inovia/Terres Univia, 2015, E-mail Communication, 17 August 2015)

Year	Location	Number of cultivars	Average erucic acid concentration (%)
2005	Atlantic Border	13	0.10
	Centre	15	0.10
	East	14	< 0.10
2006	Atlantic Border	11	< 0.10
	Centre	16	0.30
	East	11	0.10
2007	Atlantic Border	14	0.10
	Centre	14	0.10
	East	14	0.10
2008	Atlantic Border	33	< 0.10
	Centre	30	< 0.10
	East	16	< 0.10
2009	Atlantic Border	37	0.11
	Centre	35	0.10
	East	39	0.23
2010	Atlantic Border	39	0.10
	Centre	40	–
	East	40	0.10
2011	Atlantic Border	32	< 0.10
	Centre	32	0.49
	East	33	0.11
2012	Atlantic Border	18	< 0.10
	Centre	21	< 0.10
	East	18	< 0.10
2013	Atlantic Border	17	0.20
	Centre	17	0.10
	East	16	0.10
2014	Atlantic Border	15	< 0.10
	Centre	15	< 0.10
	East	15	0.25
2015	Atlantic Border	24	0.10
	Centre	27	0.10
	East	24	0.10

Appendix B – Internationally recognised protocols for fatty acid analysis based on GC of FAMEs

There is no optimal protocol for analysis of fatty acids in biological and food products. Several associations and institutions have proposed protocols that can be used for analysis of the fatty acid composition of the oil in biological samples and food products. A list of relevant methods is given below:

- Association Française de Normalisation (AFNOR): NF EN ISO 12966. Corps gras d'origines animale et végétale - Chromatographie en phase gazeuse des esters méthyliques d'acides gras
- American Oil chemists' Society (AOCS): AOCS Official Method Ce 1h-05(09). Determination of *cis*-, *trans*-, Saturated, Monounsaturated and Polyunsaturated Fatty Acids in Vegetable or Non-Ruminant Animal Oils and Fats by Capillary GLC
- American Oil chemists' Society (AOCS): AOCS Official Method Ce 1i-07(09). Determination of Saturated, *cis*-Monounsaturated, and *cis*-Polyunsaturated Fatty Acids in Marine and Other Oils Containing Long Chain Polyunsaturated Fatty Acids (PUFAs) by Capillary GLC
- American Oil chemists' Society (AOCS): AOCS Official Method Ce 1j-07(15). Determination of *cis*-, *trans*-, Saturated, Monounsaturated, and Polyunsaturated Fatty Acids in Extracted Fats by Capillary GLC
- American Oil chemists' Society (AOCS): AOCS Official Method Ce 1b-89(09). Fatty Acid Composition of Marine Oils by GLC
- AOAC International: Method 991.39. Fatty acids in encapsulated fish oils and fish oil methyl and ethyl esters
- AOAC International: Method AOAC (2012) 996.06. Fat (total, saturated, and unsaturated) in foods
- International Organization for Standardization (ISO): ISO 12966-1:2014. Animal and vegetable fats and oils – Gas chromatography of fatty acid methyl esters - Part 1: Guidelines on modern gas chromatography of fatty acid methyl esters
- International Organization for Standardization (ISO): ISO 12966-2:2011. Animal and vegetable fats and oils – Gas chromatography of fatty acid methyl esters - Part 2: Preparation of methyl esters of fatty acids
- International Organization for Standardization (ISO): ISO 12966-3:2009. Animal and vegetable fats and oils – Gas chromatography of fatty acid methyl esters – Part 3: Preparation of methyl esters using trimethylsulfonium hydroxide (TMSH)
- International Organization for Standardization (ISO): ISO 12966-4:2015. Animal and vegetable fats and oils – Gas chromatography of fatty acid methyl esters – Part 4: Determination by capillary gas chromatography
- International Organization for Standardization (ISO): ISO 6800:1997. Animal and vegetable fats and oils – Determination of the composition of fatty acids in the 2-position of the triglyceride molecules
- International Organization for Standardization (ISO): ISO/TS 17764-1:2002. Animal feeding stuffs – Determination of the content of fatty acids – Part 1: Preparation of methyl esters
- International Organization for Standardization (ISO): ISO/TS 17764-2:2002. Animal feeding stuffs – Determination of the content of fatty acids – Part 2: Gas chromatographic method
- National Standard of the People's Republic of China: GB/T 17376-2008. Animal and vegetable fats and oils - Preparation of methyl esters of fatty acids
- National Standard of the People's Republic of China: GB/T 17377-2008. Animal and vegetable fats and oils - Analysis by gas chromatography of methyl esters of fatty acids
- National Standard of the People's Republic of China: GB/T 24894-2010. Animal and vegetable fats and oils - Determination of the Composition of fatty acids in the 2-position of the triglyceride molecules
- National Standard of the People's Republic of China: GB 5413.27-2010. Determination of fatty acids in foods for infants and young children, milk and milk products
- National Standard of the People's Republic of China: GB/T 21514-2008. Determination of the content of fatty acids in feeds
- Deutsche Gesellschaft für Fettwissenschaft e.V. (DGF): C-VI 10 (13). Gaschromatographie der Fettsäuremethylester. Hinweise und Erläuterungen zu den Methoden
- Deutsche Gesellschaft für Fettwissenschaft e.V. (DGF): C-VI 10a (00). Gaschromatographie: Analyse der Fettsäuren und Fettsäureverteilung

Appendix C – Occurrence in food, human consumption data and human dietary exposure

Table C.1: Dietary surveys used for the estimation of chronic dietary exposure to erucic acid

Country	Survey acronym	Method	Survey period	No. of days per subject	No. of subjects						
					Infants	Toddlers	Other children	Adolescents *(mean age)*	Adults	Elderly	Very elderly
Austria	ASNS – Adults	24-h dietary recall	2010–2012	2	–	–	–	–	308	67	25
	ASNS – Children	24-h dietary recall	2010–2012	3	–		128	237	–	–	–
Belgium	Regional Flanders	Food record	2002–2002	3	–	36	625	–	–	–	–
Belgium	Diet National 2004	24-h dietary recall	2004	2	–	–	–	576 *(16a)*	1,292	511	704
Bulgaria	NUTRICHILD	24-h dietary recall	2007	2	861	428	433	–	–	–	–
Cyprus	Childhealth	Food record	2003	3	–	–	–	303 *(13a)*	–	–	–
Czech Republic	SISP04	24-h dietary recall	2003–2004	2	–	–	389	298 *(13a)*	1,666	–	–
Denmark	DANSDA 2005-08	Food record	2005–2008	7	–	–	298	377 *(13a)*	1,739	274	12
Denmark	IAT 2006 07	Food record	2006–2007	7	826	917	–	–	–	–	–
Finland	DIPP 2001 2009	Food record	2001–2009	3	500	500	750	–	–	–	–
Finland	NWSSP07 08	48-h dietary recall	2007–2008	4	–	–	–	306 *(13a)*	–	–	–
Finland	FINDIET2012	48-h dietary recall	2012	2	–	–	–	–	1,295	413	–
France	INCA2	Food record	2007	7	–	–	482	973 *(14a)*	2,276	264	84
Germany	VELS	Food record	2001–2002	6	159	348	293	–	–	–	–
Germany	EsKiMo	Food record	2006	3	–	–	835	393 *(11a)*	–	–	–
Germany	National Nutrition Survey II	24-h dietary recall	2007	2	–	–	–	1,011 *(16a)*	10,419	2,006	490
Greece	Regional Crete	Food record	2004–2005	3	–		838	–	–	–	–
Greece	DIET LACTATION GR	Food record	2005–2007	3	–	–	–	–	65	–	–
Hungary	National Repr Surv	Food record	2003	3	–	–	–	–	1,074	206	80
Ireland	NANS 2012	Food record	2008–2010	4	–	–	–	–	1,274	149	77
Italy	INRAN SCAI 2005 06	Food record	2005–2006	3	16	36	193	247 *(14a)*	2,313	290	228
Latvia	EFSA TEST	24-h dietary recall	2008	2	–		187	453 *(14a)*	1,271	–	–
Latvia	FC PREGNANTWOMEN 2011	24-h dietary recall	2011	2	–	–	–	–	1,002	–	–
Netherlands	VCP kids	Food record	2006–2007	3	–	322	957	–	–	–	–
Netherlands	VCPBasis AVL2007 2010	24-h dietary recall	2007–2010	2	–	–	447	1,142 *(14a)*	2,057	173	–

Country	Survey acronym	Method	Survey period	No. of days per subject	No. of subjects						
					Infants	Toddlers	Other children	Adolescents *(mean age)*	Adults	Elderly	Very elderly
Netherlands	VCP-Elderly	Food record; 24-h dietary recall	2010–2012	2	–	–	–	–	–	289	450
Romania	Dieta Pilot Adults	Food record	2012	7	–	–	–	–	1,254	83	45
Spain	enKid	24-h dietary recall	1998–2000	2	–	17	156	209 *(12a)*	–	–	–
Spain	AESAN	Food record	1999–2001	3	–	–	–	–	410	–	–
Spain	NUT INK05	24-h dietary recall	2004–2005	2	–		399	651 *(14a)*	–	–	–
Spain	AESAN FIAB	24-h dietary recall	2009	3	–	–	–	86 *(17a)*	981	–	–
Sweden	NFA	24-h dietary recall	2003	4	–	–	1,473	1,018 *(12a)*	–	–	–
Sweden	Riksmaten 2010	Food record	2010–2011	4	–	–	–	–	1,430	295	72
United Kingdom	NDNS-Rolling ProgrammeYears1-3	Food record	2008–2011	4	–	185	651	666 *(14a)*	1,266	166	139
United Kingdom	DNSIYC 2011	Food record	2011	4	1,369	1,314	–	–	–	–	–

N: number

Table C.2: Food samples excluded from the final dataset used to estimate dietary exposure and the criteria used for exclusion

Number of samples excluded	Criteria for exclusion
129	Samples identified as duplicates
423	Reported as suspect samples (not random sampling)
1,729	Samples of coconut oil, almond oil, corn oil, grape seed oil, linseed oil, olive oil, palm oil, soybean oil, sunflower oil, safflower oil, walnut oil, wheat germ oil. Erucic acid does not occur naturally in these oils
415	Samples of unspecified oil
998	Samples for which analytical results were reported on fat basis without information on the fat content, and the imputation of fat values from similar samples was not possible
178	Samples eliminated due to the application of LOQ cut-offs on key food commodities (Food for infants and Grain products)
63	Samples of composite food. They were excluded because the EFSA Comprehensive database does not differentiate between composite foods prepared at home or bought in retails/consumed outside, and this could have an important influence on the levels of erucic acid present (based on the type of oil used). Additionally, the level of disaggregation of the composite food varies from one dietary survey to another what could bias the exposure estimations They were excluded from the dataset used to estimate exposure but ad-hoc scenarios were built for these food commodities to estimate the exposure to erucic acid from the consumption of diverse types of composite food
6	Samples recodified as Feed
8	Samples of linseeds. Erucic acid does not occur naturally in linseed
1	Sample of unspecified flour. No reason to find erucic acid in this food commodity
1	Sample of boiled potatoes. No reason to find erucic acid in this food commodity
1	Sample of plain yoghurt. No reason to find erucic acid in this food commodity
1	Sample of mackerel with levels above 10% erucic acid (100 g/kg). The reported concentration most probably refers to the concentration of erucic acid in the oil or misidentification with cetoleic acid. No answer received from the data provider
1	Sample of walnut. No reason to find erucic acid in this food commodity
1	Sample of rice. No reason to find erucic acid in this food commodity
2	Samples of custard as no food commodities containing erucic acid is usually used in the elaboration of this dessert

EFSA: European Food Safety Authority; LOQ: limit of quantification.

Table C.3: Use of cut-offs for the limits of quantification (LOQs) of selected food groups and its effect on the final occurrence values

	Number samples before applying cut-off	LC %	Mean occurrence values before application of cut-offs (mg/kg)		% difference between LB/UB	Cut-off applied on LOQ	Number samples excluded	Number samples after applying cut-off	Mean occurrence values after application of cut-offs (mg/kg)		% difference between LB/UB
			LB	UB					LB	UB	
Pastries and cakes	800	55	220	347	60	600	67	733	237	290	20
Biscuits (cookies)	201	81	212	491	130	1,300	44	157	272	393	40
Ready-to-eat meal for infants and young children	194	44	90	253	180	125	38	156	77	86	10

LB: lower bound; LC: left-censored; LOQ: limit of quantification; UB: upper bound.

Table C.4: Samples of composite food excluded from final dataset

Food groups[(a)]	Number of samples	LB/UB	Mean concentration (mg/kg)
Composite food (including frozen products), unspecified	7	LB	703
		UB	708
Cereal-based dishes, unspecified	2	LB	0
		UB	94
Pasta, cooked	3	LB	2,667
		UB	2,667
Rice-based meals	1	LB	67,300
		UB	67,300
Meat-based meals	18	LB	429
		UB	442
Fish and seafood based meals	1	LB	5,000
		UB	5,000
Ready-to-eat soups	28	LB	24
		UB	77
Prepared salads	3	LB	2,141
		UB	2,141

LB: lower bound; UB: upper bound.
(a): The food groups in bold refer to FoodEx level 2 and the other food groups to FoodEx level 3.

Table C.5: Erucic acid occurrence values in different food commodities (mg/kg), grouped at different FoodEx levels (FoodEx link) depending on their occurrence values before estimating dietary exposure

FoodEx 1 level	FoodEx link[(a)]	N	% LC	Mean concentration (mg/kg)			95th percentile concentration (mg/kg)		
				LB	MB	UB	LB	MB	UB
Grains and grain-based products-	Grain milling products, unspecified	5	100	0	38	76	0	150	300
	Bread and rolls	43	56	42	52	63	–	–	–
	Pasta (Raw)	2	50	36	38	41	–	–	–
	Breakfast cereals	30	50	73	79	84	–	–	–
	Fine bakery wares, unspecified	12	42	650	671	691	–	–	–
	Pastries and cakes	733	50	237	263	290	1,100	1,100	1,100
	Biscuits (cookies)	157	76	272	332	393	1,800	1,800	1,800
Vegetables and vegetable products (including fungi)	Brassica vegetables	1	100	0	166	331	–	–	–
	Mustard seedling (Sinapis alba)	1	0	179,000	179,000	179,000	–	–	–
	Lamb's lettuce (Valerianella locusta)	1	100	0	0	1	–	–	–
	Cocoa beans and cocoa products	27	100	0	88	176	–	–	–
	Vegetable products, unspecified	1	100	0	7	14	–	–	–

FoodEx 1 level	FoodEx link[(a)]	N	% LC	Mean concentration (mg/kg)			95th percentile concentration (mg/kg)		
				LB	MB	UB	LB	MB	UB
Starchy roots and tubers	*Potatoes and potatoes products, unspecified*	34	53	320	331	342	–	–	–
	French fries	1,178	38	283	292	300	859	859	859
	Potato fried	4	25	170	174	178	–	–	–
	Potato croquettes	5	60	92	122	151	–	–	–
	Potato boiled	2	100	0	7	14	–	–	–
Legumes, nuts and oilseeds	*Soybeans (Glycine max)*	2	100	0	32	63	–	–	–
	Peanut (Arachis hypogea)	28	11	1,703	1,709	1,716	–	–	–
	Tree nuts	108	100	0	57	113	0	100	200
	Oilseeds, unspecified	68	100	0	56	113	–	–	–
	Rape seed (Brassica napus)	2	50	1,500	2,750	4,000	–	–	–
	Poppy seed (Papaver somniferum)	2	100	0	80	160	–	–	–
	Sesame seed (Sesamum indicum syn. S. orientale)	6	100	0	50	101	–	–	–
	Sunflower seed (Helianthus annuus)	8	100	0	50	99	–	–	–
	Pumpkin seeds (Cucurbita pepo var. oleifera)	1	100	0	47	93	–	–	–
Fruit and fruit products	*Stone fruits*	1	100	0	50	100	–	–	–
	Miscellaneous fruits	2	100	0	14	29	–	–	–
	Dried fruits	1	100	0	500	1,000	–	–	–
Meat and meat products (including edible offal)	Livestock meat	2	100	0	500	1,000	–	–	–
	Textured soy protein	11	0	934	934	934	–	–	–
Fish and other seafood (including amphibians, reptiles, snails and insects)	*Fish meat, unspecified*	1	0	76	76	76	–	–	–
	Herring (Clupea)	3	0	3,560	3,560	3,560	–	–	–
	Anchovy (Engraulis)	1	100	0	30	60	–	–	–
	Sardine and pilchard (Sardina)	14	29	858	933	1,009	–	–	–
	Salmon and trout (Salmo spp.)	9	0	992	992	992	–	–	–
	Mackeral (Scomber)	1	0	370	370	370	–	–	–
	Tuna (Thunnus)	18	94	7	40	73	–	–	–
	Other fish offal (not fish roe)	1	0	6,768	6,768	6,768	–	–	–
	Octopus	2	100	0	6	11	–	–	–

FoodEx 1 level	FoodEx link[a]	N	% LC	Mean concentration (mg/kg)			95th percentile concentration (mg/kg)		
				LB	MB	UB	LB	MB	UB
Milk and dairy products	Milk and dairy products, unspecified	17	53	125	149	173	–	–	–
	Cow milk	26	100	0	113	225	–	–	–
	Flavoured milk	1	100	0	1	2	–	–	–
	Concentrated milk	21	100	0	136	271	–	–	–
	Whey and whey products	2	100	0	113	225	–	–	–
	Cream and cream products	222	100	1	146	290	0	150	300
	Fermented milk products, unspecified	4	50	363	368	373	–	–	–
	Cheese, unspecified	21	81	88	158	229	–	–	–
	Quark	6	100	0	138	275	–	–	–
	Cheese, processed, sliceable	43	70	170	222	273	–	–	–
	Cheese, processed spreadable	14	86	115	243	371	–	–	–
	Cheese, processed cheese, plain	5	100	0	67	134	–	–	–
	Cheese, Appenzeller	3	100	0	181	362	–	–	–
	Cheese, Edam	9	100	0	142	283	–	–	–
	Cheese, Blue Castello	1	100	0	150	300	–	–	–
	Cheese, Camembert	2	100	0	150	300	–	–	–
	Cheese, Cheddar	1	100	0	150	300	–	–	–
	Cheese, Emmental	7	100	0	167	335	–	–	–
	Cheese, Maasdam	3	100	0	150	300	–	–	–
	Cheese, Mozzarella	4	100	0	150	300	–	–	–
	Cheese, Ricotta	1	100	0	150	300	–	–	–
	Cheese, Smoked Gouda	1	100	0	75	150	–	–	–
	Cheese, Feta	35	66	212	276	339	–	–	–
	Cheese, Gouda	14	79	141	252	363	–	–	–
	Cheese, Trappist	23	96	57	193	330	–	–	–
	Milk and milk product imitates	18	72	114	209	304	–	–	–
Eggs and egg products	Eggs and egg products, unspecified	3	100	0	11	21	–	–	–
	Eggs, fresh	44	100	0	34	68	–	–	–
	Eggs, powder	12	100	0	150	300	–	–	–

FoodEx 1 level	FoodEx link[a]	N	% LC	Mean concentration (mg/kg)			95th percentile concentration (mg/kg)		
				LB	MB	UB	LB	MB	UB
Sugar and confectionary	Sugar and confectionary, unspecified	12	75	198	228	259	–	–	–
	Chocolate (Cocoa) products	39	100	0	101	201	–	–	–
	Bitter chocolate	29	100	0	134	269	–	–	–
	Chocolate coated confectionery	17	100	0	133	267	–	–	–
	Filled chocolate	13	100	0	150	300	–	–	–
	White chocolate	9	100	0	201	401	–	–	–
	Chocolate substitutes	8	100	0	131	263	–	–	–
	Chocolate, cream	32	63	173	213	253	–	–	–
	Milk chocolate	150	99	7	128	250	0	150	300
	Pralines	52	98	2	101	201	–	–	–
	Confectionery (non-chocolate)	9	100	0	196	392	–	–	–
Animal and vegetable fats and oils	Animal and vegetable fats and oils, unspecified	2	50	2,533	2,783	3,033	–	–	–
	Animal fat, unspecified	8	100	0	448	896	–	–	–
	Duck fat	1	100	0	500	1,000	–	–	–
	Tallow	4	100	0	500	1,000	–	–	–
	Butter	67	88	38	204	370	200	413	826
	Butter oil	3	67	198	298	398	–	–	–
	Pork lard (Schmaltz)	30	93	50	401	751	–	–	-
	Fish oil, unspecified	3	0	7,000	7,000	7,000	–	–	–
	Cod liver oil	1	0	14,000	14,000	14,000	–	–	–
	Vegetable fat, unspecified	36	56	492	600	708	–	–	–
	Peanuts butter	10	70	203	536	869	–	–	–
	Coconut fat	14	100	0	307	614	–	–	–
	Palm fat	13	100	0	475	950	–	–	–
	Cocoa butter	1	100	0	150	300	–	–	–
	Vegetable oil unspecified	415	67	5,026	5,325	5,623	6,600	6,600	6,600
	Oil, frying, blend	250	82	235	655	1,075	1,000	1,000	1,000
	Peanut oil	96	29	1,884	2,014	2,144	5,500	5,500	5,500
	Rapeseed oil	5,832	81	1,285	3,250	5,215	7,400	7,560	7,560
	Fats of mixed origin	181	40	985	1,031	1,078	3,800	3,800	3,800
	Margarine and similar products, unspecified	343	40	663	742	820	1,979	1,979	1,979
	Margarine, normal fat	29	31	877	922	968	–	–	–
	Margarine, low fat	42	19	662	670	679	–	–	–
	Margarine with other ingredients	69	13	1,422	1,438	1,454	3,900	3,900	3,900
	Fat emulsions	14	36	805	867	929	–	–	–
Alcoholic beverages	*Coffee liqueur*	3	67	3	6	9	–	–	–
	Egg liqueur	7	100	0	2	5	–	–	–
	Cream liqueur	8	100	0	12	24	–	–	–

FoodEx 1 level	FoodEx link[a]	N	% LC	Mean concentration (mg/kg)			95th percentile concentration (mg/kg)		
				LB	MB	UB	LB	MB	UB
Drinking water	Drinking water	4	100	0	500	1,000	–	–	–
Herbs, spices and condiments	Herbs, spices and condiments, unspecified	5	20	627	636	645	–	–	–
	Spices undefined	2	100	0	53	107	–	–	–
	Paprika powder	1	0	2,000	2,000	2,000	–	–	–
	Chilli powder	1	100	0	67	133	–	–	–
	Herb and spice mixtures	2	100	0	1,000	2,000	–	–	–
	Mixed spices	3	67	62,000	62,177	62,355	–	–	–
	Seasoning or extracts, unspecified	1	100	0	47	94	–	–	–
	Stock cubes (bouillon cube)	2	100	0	89	177	–	–	–
	Gravy instant granules	25	96	38	140	242	–	–	–
	Vegetable extracts	2	100	0	7	13	–	–	–
	Condiment unspecified	6	67	1,881	2,059	2,236	–	–	–
	Mustard, sweet	8	0	10,433	10,433	10,433	–	–	–
	Mustard, mild	9	11	14,081	14,082	14,083	–	–	–
	Mustard, hot	51	0	19,712	19,712	19,712	–	–	–
	Horseradish sauce	1	0	656	656	656	–	–	–
	Curry sauce	1	100	0	29	58	–	–	–
	Tartar sauce	2	50	1,300	1,500	1,700	–	–	–
	Dressing	168	5	2,266	2,269	2,272	5,600	5,600	5,600
	Savoury sauces, unspecified	16	31	12	33	53	–	–	–
	White sauce (Bechamel sauce, Cheese sauce)	2	50	53	87	120	–	–	–
	Emulsion sauce (Hollandaise sauce)	13	77	243	332	421	–	–	–
	Oil-based sauce (Pesto, Aioli sauce)	16	94	4	74	145	–	–	–
	Vegetable sauce	3	100	0	14	29	–	–	–
	Flavourings or essences	1	100	0	150	300	–	–	–
	Glaze	1	100	0	97	194	–	–	–
Food for infants and small children	Food for infants and small children, unspecified	79	51	168	262	356	790	790	1,000
	Infant formulae, powder	218	32	220	253	286	552	552	553
	Follow-on formulae, powder	191	51	142	187	232	430	483	483
	Cereal-based food for infants and young children	166	44	128	145	161	620	620	620
	Ready-to-eat meal for infants and young children	156	31	77	81	86	281	281	281

FoodEx 1 level	FoodEx link[(a)]	N	% LC	Mean concentration (mg/kg)			95th percentile concentration (mg/kg)		
				LB	MB	UB	LB	MB	UB
Products for special nutritional use	Products for special nutritional use, unspecified	2	100	0	500	1,000	–	–	–
	Food for weight reduction	23	65	63	79	95	–	–	–
	Dietary supplements, unspecified	22	32	12,150	12,286	12,423	–	–	–
	Mineral supplements	1	100	0	17	35	–	–	–
	Protein and amino acids supplements	1	100	0	150	300	–	–	–
	Supplements containing special fatty acids (e.g. omega-3, essential fatty acids)	26	12	4,237	4,280	4,322	–	–	–
	Plant extract formula	1	0	4	4	4	–	–	–
	Algae formula (e.g. Spirulina, Chlorella)	7	14	1,014	1,021	1,029	–	–	–
	Pollen-based supplement	1	0	328	328	328	–	–	–
	Dietetic food for diabetics (labelled as such)	47	91	51	114	176	–	–	–
	Medical food	75	31	650	706	762	2,000	2,000	2,000
Snacks, desserts, and other foods	*Snack food, unspecified*	12	100	0	34	68	–	–	–
	Potato crisps	67	90	27	80	133	100	100	200
	Pretzels	1	100	0	18	35	–	–	–
	Ice and desserts, unspecified	19	89	101	121	141	–	–	–
	Ice cream, milk-based	325	96	1	33	65	0	92	164
	Ice cream, not milk-based	110	97	0	26	51	0	54	108
	Starchy pudding	18	78	398	450	502	–	–	–
	Sorbet	1	10	0	150	300	–	–	–
	Other foods (foods which cannot be included in any other group)	3	67	308	367	426	–	–	–

LB: lower bound; LC: left-censored; MB: middle bound; N: number; UB: upper bound.
(a): This column explains how the different food commodities were grouped at different FoodEx levels depending on the reported occurrence values: FoodEx Level 2 (non-italics), FoodEx level 3 (italics).

Table C.6: Mean and 95th percentile chronic dietary exposure to erucic acid (mg/kg bw per day) for total population in lower bound and upper bound scenarios

Dietary surveys[b]	Range of dietary exposure (LB – UB) (mg/kg bw per day)													
	Infants		Toddlers		Other children		Adolescents		Adults		Elderly		Very elderly	
	Mean	P95	Mean	P95	Mean	P95	Mean	P95	Mean	P95	Mean	P95	Mean	P95
ASNS - Adults									0.9–1.2	2.7–3.1	0.8–1.0	2.0–2.4	0.9–1.2	–[a]
ASNS - Children					1.8–3.7	4.0–9.5	1.0–2.1	2.4–5.4						
Regional Flanders			2.8–4.4	–[a]	2.2–3.1	4.6–6.7								
Diet National 2004							1.3–1.7	3.0–3.5	1.2–1.5	3.1–3.5	1.1–1.4	2.7–3.0	1.1–1.4	2.8–3.3
NUTRICHILD	2.2–3.0	5.8–7.4	1.7–2.7	4.4–5.9	1.7–2.6	4.7–6.0								
Childhealth							0.6–0.8	1.2–1.7						
SISP04					1.6–2.4	5.3–6.1	1.6–2.2	4.8–5.4	1.2–1.5	3.8–4.3				
DANSDA 2005-08					2.0–2.7	4.0–5.1	0.9–1.3	2.0–2.6	0.8–1.1	1.7–2.2	0.9–1.1	1.7–2.1	1.0–1.3	–[a]
IAT 2006 07	1.8–2.5	3.5–4.8	2.0–2.8	3.9–4.9										
DIPP 2001 2009	0.7–1.7	1.7–4.5	1.3–2.8	3.2–6.5	1.9–3.1	4.6–6.6								
NWSSP07 08							1.0–1.3	2.2–2.7						
FINDIET2012									1.5–1.9	3.7–4.1	1.4–1.8	3.2–3.8		
INCA2					2.1–3.1	4.1–5.5	1.2–1.6	2.4–3.3	1.0–1.3	2.1–2.6	0.9–1.3	2.0–2.7	0.9–1.3	2.0–2.6
VELS	2.8–3.7	5.0–7.0	1.9–2.8	3.8–5.3	1.7–2.5	3.3–4.4								
EsKiMo					1.3–1.9	3.1–3.9	1.0–1.5	2.5–3.2						
National Nutrition Survey II							0.8–1.2	2.4–2.9	1.0–1.3	2.8–3.3	1.0–1.3	2.8–3.3	1.0–1.4	2.8–3.3
Regional Crete					1.2–1.7	2.7–3.4								
DIET LACTATION GR									0.6–0.8	1.4–1.7				
National Repr Surv									1.0–1.3	3.2–3.5	0.8–1.0	2.1–2.5	0.7–1.0	2.7–3.1
NANS 2012									1.0–1.3	2.1–2.6	1.1–1.4	2.7–3.1	1.1–1.3	2.2–2.5
INRAN SCAI 2005 06	1.8–2.4	–[a]	1.4–2.2	–[a]	1.0–1.6	2.1–3.1	0.6–1.0	1.3–2.1	0.4–0.6	1.0–1.4	0.3–0.6	1.0–1.3	0.4–0.6	1.1–1.4
EFSA TEST					1.2–1.8	3.2–4.7	1.0–1.4	2.4–3.4	0.7–1.0	2.0–2.6				
FC PREGNANTWOMEN 2011									1.0–1.4	2.9–3.3				
VCP kids			2.0–3.1	4.3–6.0	1.9–2.8	4.0–5.4								
VCPBasis AVL2007 2010					2.4–3.2	5.0–6.7	1.6–2.1	3.6–4.5	1.2–1.6	2.9–3.5	1.1–1.4	3.1–3.5		
VCP-Elderly											1.1–1.4	2.7–3.4	1.1–1.4	2.9–3.3
Dieta Pilot Adults									0.3–0.6	0.9–1.3	0.3–0.5	0.7–1.1	0.4–0.7	–[a]

Dietary surveys[b]	Range of dietary exposure (LB – UB) (mg/kg bw per day)													
	Infants		Toddlers		Other children		Adolescents		Adults		Elderly		Very elderly	
	Mean	P95	Mean	P95	Mean	P95	Mean	P95	Mean	P95	Mean	P95	Mean	P95
enKid			1.2–1.7	–[a]	1.4–1.8	3.4–4.1	1.0–1.3	2.4–2.9						
AESAN									0.5–0.7	1.4–1.9				
NUT INK05					1.0–1.5	2.3–3.1	0.7–1.0	1.5–2.1						
AESAN FIAB							0.5–0.8	1.1–1.8	0.5–0.7	1.3–1.7				
NFA					1.7–2.2	4.0–4.7	1.0–1.3	2.4–2.8						
Riksmaten 2010									1.1–1.5	2.8–3.4	1.3–1.6	3.9–4.2	1.3–1.6	3.2–3.5
NDNS-RollingProgrammeYears1-3			2.1–2.9	4.4–5.5	1.9–2.5	3.8–4.7	1.0–1.3	2.1–2.6	0.8–1.0	1.7–2.0	0.9–1.1	2.0–2.3	0.9–1.1	2.1–2.7
DNSIYC 2011	1.8–2.6	4.1–5.7	2.0–2.9	4.1–5.6										

bw: body weight; LB: lower bound; P95: 95th percentile; UB: upper bound.
(a): The 95th percentile with less than 60 observations may not be statistically robust (EFSA, 2011b). Those estimates were not included in this table.
(b): Details on the dietary surveys and the number of subjects are given in Appendix C, Table C.1.

Table C.7: Average contribution of food commodities grouped at FoodEx Level 1 to the total average chronic dietary exposure to erucic acid

Age class	FoodEx level 1	Number of dietary surveys (% average contribution under the MB scenario)					
		< 1%	1–5%	5–10%	10–25%	25–50%	50–75%
Infants	Grains and grain-based products	–	1	1	4	–	–
	Starchy roots and tubers	5	1	–	–	–	–
	Legumes, nuts and oilseeds	6	–	–	–	–	–
	Meat and meat products (including edible offal)	6	–	–	–	–	–
	Fish and other seafood (including amphibians, reptiles, snails and insects)	2	2	1	1	–	–
	Milk and dairy products	–	3	1	2	–	–
	Sugar and confectionary	6	–	–	–	–	–
	Animal and vegetable fats and oils	1	1	2	–	1	1
	Alcoholic beverages	6	–	–	–	–	–
	Herbs, spices and condiments	4	2	–	–	–	–
	Food for infants and small children	–	1	–	–	1	4
	Products for special nutritional use	3	1	1	1	–	–
	Snacks, desserts, and other foods	4	2	–	–	–	–
Toddlers	Grains and grain-based products	–	–	–	2	7	1
	Starchy roots and tubers	4	3	1	2	–	–
	Legumes, nuts and oilseeds	10	–	–	–	–	–
	Meat and meat products (including edible offal)	10	–	–	–	–	–
	Fish and other seafood (including amphibians, reptiles, snails and insects)	1	6	1	2	–	–
	Milk and dairy products	–	–	1	8	1	–
	Sugar and confectionary	6	4	–	–	–	–
	Animal and vegetable fats and oils	–	2	2	3	2	1
	Alcoholic beverages	10	–	–	–	–	–
	Herbs, spices and condiments	3	3	3	1	–	–
	Food for infants and small children	1	4	2	2	1	–
	Products for special nutritional use	5	1	3	1	–	–
	Snacks, desserts, and other foods	4	4	–	2	–	–

Age class	FoodEx level 1	Number of dietary surveys (% average contribution under the MB scenario)					
		< 1%	1–5%	5–10%	10–25%	25–50%	50–75%
Other children	Grains and grain-based products	–	1	–	1	12	4
	Starchy roots and tubers	2	5	5	6	–	–
	Legumes, nuts and oilseeds	10	8	–	–	–	–
	Meat and meat products (including edible offal)	18	–	–	–	–	–
	Fish and other seafood (including amphibians, reptiles, snails and insects)	2	11	3	2	–	–
	Milk and dairy products	–	–	7	11	–	–
	Sugar and confectionary	9	9	–	–	–	–
	Animal and vegetable fats and oils	–	4	2	9	3	–
	Alcoholic beverages	18	–	–	–	–	–
	Herbs, spices and condiments	–	3	7	8	–	–
	Food for infants and small children	13	5	–	–	–	–
	Products for special nutritional use	12	1	5	–	–	–
	Snacks, desserts, and other foods	–	13	3	2	–	–
Adolescents	Grains and grain-based products	–	1	–	1	12	3
	Starchy roots and tubers	2	6	2	6	1	–
	Legumes, nuts and oilseeds	6	11	–	–	–	–
	Meat and meat products (including edible offal)	17	–	–	–	–	–
	Fish and other seafood (including amphibians, reptiles, snails and insects)	2	9	3	3	–	–
	Milk and dairy products	–	3	9	5	–	–
	Sugar and confectionary	7	10	–	–	–	–
	Animal and vegetable fats and oils	–	4	2	8	3	–
	Alcoholic beverages	17	–	–	–	–	–
	Herbs, spices and condiments	–	1	5	8	3	–
	Food for infants and small children	17	–	–	–	–	–
	Products for special nutritional use	12	2	3	–	–	–
	Snacks, desserts, and other foods	2	13	2	–	–	–

Age class	FoodEx level 1	Number of dietary surveys (% average contribution under the MB scenario)					
		< 1%	1–5%	5–10%	10–25%	25–50%	50–75%
Adults	Grains and grain-based products	–	–	–	6	11	–
	Starchy roots and tubers	4	8	–	5	–	–
	Legumes, nuts and oilseeds	2	14	1	–	–	–
	Meat and meat products (including edible offal)	15	2	–	–	–	–
	Fish and other seafood (including amphibians, reptiles, snails and insects)	–	2	7	8	–	–
	Milk and dairy products	–	1	9	6	1	–
	Sugar and confectionary	14	3	–	–	–	–
	Animal and vegetable fats and oils	–	3	3	10	1	–
	Alcoholic beverages	17	–	–	–	–	–
	Herbs, spices and condiments	–	1	1	9	5	1
	Food for infants and small children	17	–	–	–	–	–
	Products for special nutritional use	12	1	3	1	–	–
	Snacks, desserts, and other foods	6	11	–	–	–	–
Elderly	Grains and grain-based products	–	–	–	7	7	–
	Starchy roots and tubers	4	5	4	1	–	–
	Legumes, nuts and oilseeds	9	5	–	–	–	–
	Meat and meat products (including edible offal)	13	–	1	–	–	–
	Fish and other seafood (including amphibians, reptiles, snails and insects)	1	1	1	9	2	–
	Milk and dairy products	–	1	8	4	1	–
	Sugar and confectionary	14	–	–	–	–	–
	Animal and vegetable fats and oils	–	1	1	10	2	–
	Alcoholic beverages	14	–	–	–	–	–
	Herbs, spices and condiments	1	1	2	7	3	–
	Food for infants and small children	14	–	–	–	–	–
	Products for special nutritional use	8	1	1	4	–	–
	Snacks, desserts, and other foods	8	6	–	–	–	–

Age class	FoodEx level 1	Number of dietary surveys (% average contribution under the MB scenario)					
		< 1%	1–5%	5–10%	10–25%	25–50%	50–75%
Very elderly	Grains and grain-based products	–	–	–	6	6	–
	Starchy roots and tubers	3	7	1	1	–	–
	Legumes, nuts and oilseeds	11	1	–	–	–	–
	Meat and meat products (including edible offal)	11	1	–	–	–	–
	Fish and other seafood (including amphibians, reptiles, snails and insects)	–	2	1	7	2	–
	Milk and dairy products	–	2	7	3	–	–
	Sugar and confectionary	12	–	–	–	–	–
	Animal and vegetable fats and oils	–	1	–	9	2	–
	Alcoholic beverages	12	–	–	–	–	–
	Herbs, spices and condiments	1	1	4	4	2	–
	Food for infants and small children	12	–	–	–	–	–
	Products for special nutritional use	7	1	–	4	–	–
	Snacks, desserts, and other foods	6	6	–	–	–	–
Pregnant women	Grains and grain-based products	–	–	–	–	1	–
	Starchy roots and tubers	–	–	1	–	–	–
	Legumes, nuts and oilseeds	1	–	–	–	–	–
	Meat and meat products (including edible offal)	1	–	–	–	–	–
	Fish and other seafood (including amphibians, reptiles, snails and insects)	–	–	–	1	–	–
	Milk and dairy products	–	–	–	1	–	–
	Sugar and confectionary	1	–	–	–	–	–
	Animal and vegetable fats and oils	–	1	–	–	–	–
	Alcoholic beverages	1	–	–	–	–	–
	Herbs, spices and condiments	–	–	–	1	–	–
	Food for infants and small children	1	–	–	–	–	–
	Products for special nutritional use	1	–	–	–	–	–
	Snacks, desserts, and other foods	1	–	–	–	–	–

Age class	FoodEx level 1	Number of dietary surveys (% average contribution under the MB scenario)					
		< 1%	1–5%	5–10%	10–25%	25–50%	50–75%
Lactating women	Grains and grain-based products	–	–	–	–	–	1
	Starchy roots and tubers	–	1	–	–	–	–
	Legumes, nuts and oilseeds	1	–	–	–	–	–
	Meat and meat products (including edible offal)	1	–	–	–	–	–
	Fish and other seafood (including amphibians, reptiles, snails and insects)	–	1	–	–	–	–
	Milk and dairy products	–	–	–	1	–	–
	Sugar and confectionary	1	–	–	–	–	–
	Animal and vegetable fats and oils	–	–	1	–	–	–
	Alcoholic beverages	1	–	–	–	–	–
	Herbs, spices and condiments	–	1	–	–	–	–
	Food for infants and small children	1	–	–	–	–	–
	Products for special nutritional use	1	–	–	–	–	–
	Snacks, desserts, and other foods	–	1	–	–	–	–

MB: middle bound.

Appendix D – Feed Intake and composition of diets used in estimating animal exposure to erucic acid

Estimates of exposure require information on both the amount of feed consumed and levels of erucic acid in the feed. This Appendix gives feed intakes (on a per day basis) for different categories of farmed livestock and fish that have been assumed in this Scientific Opinion to estimate exposure to erucic acid.

Both the amount of feed consumed and the composition of the diets vary considerably, both between and within animal categories, within the European Union (EU). In this Opinion, the composition of diets for each of the major farm livestock species are based on published guidelines on nutrition and feeding (e.g. Carabano and Piquer, 1998; NRC 2007a,b; Leeson and Summers, 2008; McDonald et al., 2011; EFSA FEEDAP Panel, 2012; OECD, 2013) and data on the EU manufacture of compound feeds.[51] They are therefore estimates of the Panel on Contaminants in the Food Chain (CONTAM Panel), but are in agreement with common practice.

In estimating exposure, it has been assumed that erucic acid is only present in rapeseed meal and rapeseed oil. The amounts of these feeds included in the diets of farmed and companion animals also vary. In the absence of any European data on levels of rapeseed meal fed, the CONTAM Panel have adopted the maximum levels recommended by a number of sources (Ewing, 1997; Cottrill et al., 2007; Canola Council of Canada, 2015) for the different animal categories. Similarly for rapeseed oil, industry recommendations for the maximum inclusion rates have been adopted. In practice, the amounts fed are likely to be lower than recommended maxima; rapeseed oil is often part of a mix of vegetable oils, with inclusion rates determined by the prices of the different oils. However, by adopting this approach likely worst-case exposures have been calculated.

The levels of erucic acid in rapeseed meal and oil are given in Table 5 (Section 3.1.2.2). Based on these, and estimates of feed intake given below, the lower bound (LB) and upper bound (UB) exposure estimates of erucic acid were calculated for the farm livestock species, and are given in this Appendix.

Information provided to EFSA by the European Pet Food Industry Federation (FEDIAF) indicated that meal, rapeseed oil and meals and oils from other Brassicaceae crops are not common constituents of cat and dog foods (FEDIAF, Personal communication by email, May 2016). Therefore, no exposure assessments have been undertaken for these animals.

D.1. Feed intakes

D.1.1. Ruminants and horses

The diets of cattle, sheep and goats consist predominantly of forages supplemented mainly with cereal grains and vegetable proteins and other by-products of food production as necessary. In some situations, forages may represent the total diet.

For this scientific opinion, exposure has been estimated for a 650-kg dairy cow, with a milk yield of 40 kg/day (considered as a high milk yield), and where non-forage feeds account for 40% of the total diet. For many beef cattle, forage will comprise the total ration, but where higher growth rates or physiological status require it, additional non-forage feeds will be provided. The amount of feed consumed varies considerably between systems, and depends on the breed and live weight of the animal, the amount and quality of the feeds available and the intended rate of live weight gain and body condition. In this opinion, exposure estimates are given for fattening beef cattle with live weights of 400 kg and a daily live weight gain of 1.0 kg.

The diets of sheep and goats reared for meat production consist predominantly of forage, with additional non-forage feeds given when high levels of live weight gain are required. Total daily dry matter (DM) intakes can range from 1.9% to 3.8% of their body weight (Devendra and Burns, 1983), of which forages typically account for 75% or more of total intake. Goats reared for meat production and with a body weight > 10 kg are often fed green fodder *ad libitum* (AFRC, 1993) supplemented with cereal grains (barley, oats or maize), cereal by-products and vegetable proteins (McDonald et al., 2011).

For milking sheep and goats, compound feeding usually commences in late pregnancy and continues into lactation. Due to physiological and metabolic differences, goats are able to consume

[51] www.fefac.eu

diets with higher proportions of non-forage feeds without adversely affecting feed intake or production (Avondo et al., 2008), but the actual amounts fed depend on the quality of the forage available. Non-lactating animals usually receive only forage feeds.

The live weights, feed intakes, the proportion of the daily ration that is non-forage feed and growth rates/productivity for cattle, sheep, goats and horses used in this Scientific Opinion are given in Table D.1.

Table D.1: Live weights, growth rate/productivity, dry matter intake for cattle, sheep, goats and horses, and the proportions of the diet as non-forage

	Live weight (kg)	Growth rate or productivity	Dry matter intake (kg/day)	% diet as non-forage feed	Reference
Dairy cows, lactating	650	40 kg milk/day	20.7	40	AFRC (1993)
Fattening cattle: beef [(a)]	400	1 kg/day	9.6	15	AFRC (1993)
Sheep: lactating	80	Feeding twin lambs	2.8	50	AFRC (1993)
Goats: milking[(b)]	60	6 kg milk/day	3.4	65	NRC (2007a)
Goats: fattening	40	0.2 kg/day	1.5	40	NRC (2007a)
Horses	450	Moderate activity	9.0	50	NRC (2007b)

(a): Housed castrate cattle, medium maturing breed.
(b): Months 2–3 of lactation.

D.1.2. Pigs, poultry and farmed fish

Exposure estimates for this Opinion have been made for piglets (20 kg bw), fattening pigs (100 kg bw) and lactating sows (200 kg bw) using feed intakes proposed by EFSA (2009).

The amount of feed voluntarily consumed by poultry is largely determined by the size and age of the bird and the production system (rearing, fattening or laying). Under *ad libitum* feeding, daily intake increases as the birds get older, although relative to body weight it declines with age. For meat producing and egg-laying birds, *ad libitum* feeding is widely practiced, but for breeding stock feed intake is frequently restricted to maintain a steady body weight (Leeson and Summers, 2008).

Commercially reared species of farmed fish include Atlantic salmon, rainbow trout, sea bass, sea bream, cod, halibut, tuna, eel and turbot. Traditionally, the principal raw materials used for the manufacture of fish feeds in Europe have been fishmeal and fish oils, and although alternative sources of oil and protein are increasingly being used fish-derived feeds still remain the major ingredients.

Data for feed intake and live weight of pigs, poultry and farmed fish are from EFSA FEEDAP Panel (2012) and of ducks from Leeson and Summers (2008) are used in this Scientific Opinion (Table D.2).

Table D.2: Live weights and feed intake for pigs, poultry and fish

	Live weight (kg)	Feed intake (kg dry matter/day)
Pigs: piglets	20	1.0
Pigs: fattening pigs	100	3.0
Pigs: lactating sows	200	6.0
Poultry: broilers[(a)]	2	0.12
Poultry: laying hens	2	0.12
Turkeys: fattening turkeys	12	0.40
Ducks: fattening ducks	3	0.14
Salmonids	2	0.04

(a): Chickens for fattening.

D.1.3. Rabbits

Commercial rabbit production takes place in at least 14 EU Member States, with the largest producers being Italy, France and Spain. Rabbits are usually fed a pelleted diet of dried forages, cereals and vegetable proteins supplemented with minerals, vitamins and trace elements. A daily intake of 75 g/kg bw for a 2-kg rabbit is used in this Scientific Opinion to estimate exposure (derived from Carabano and Piquer, 1998).

D.1.4. Farmed mink

For estimating exposure, the CONTAM Panel has assumed a live weight of 2.07 kg for a male mink at pelting, and with a feed intake of 227 g/day (75 g DM) (NRC, 1982).

D.2. Diet composition and estimates of erucic acid concentration in diets of farmed livestock and fish

Many livestock in the EU are fed proprietary commercial compound feeds and for some, particularly non-ruminant animals, they are often the sole feed. However, insufficient data have been provided on levels of erucic acid in species-specific compound feeds, and therefore, it has been necessary to estimate exposure using inclusion rates of rapeseed meal and rapeseed oil in the daily rations. These data are given below.

D.2.1. Ruminants and horses

As described above, forages are major constituents of ruminant diets, but erucic acid has not been reported in these feeds and it has therefore been assumed that they make no contribution to exposure. For all categories of ruminants, rapeseed cakes/meals are extremely important sources of protein, and inclusion levels of 25% or more in the non-forage part of the ration are not uncommon. Vegetable oils are also frequently added to ruminant rations, although inclusion rates are normally restricted to < 5% because of the potential adverse effects of the oils on rumen fermentation. Since this normally consists of a blend of vegetable oils, an inclusion rate of 2% in the non-forage part of the ration has been assumed. In sheep and goat rations, rapeseed meal should not exceed 20% of their total non-forage ration (Ewing, 1997).

There has been relatively little research on optimum levels of rapeseed meal in diets for horses. Sutton (1988) reported that up to 15% canola meal, the highest level tested, in recreational horse diets had no adverse effect on feed intake, and this value has been used to assess exposure to erucic acid from rapeseed meal.

Table D.3 gives assumed levels of rapeseed meal and rapeseed oil in the non-forage part of ruminant and horse diets, but it should be noted that estimated mean LB and UB concentrations of erucic acid refer to the whole diet, i.e. they take account of the relative proportions of forage and non-forage feeds in the diet (as given in Table D.1).

Table D.3: Assumed inclusion rates of rapeseed meal and rapeseed oil in the non-forage part of diets for ruminants and horses, and calculated mean lower bound and upper bound concentrations of erucic acid in the total diet

Feed materials	Lactating dairy cows	Fattening beef cattle	Lactating sheep	Dairy goats	Fattening goats	Horses
Rapeseed meal (%)	25	25	20	20	20	15
Rapeseed oil (%)	2	2	2	2	2	2
Estimated mean erucic acid concentration						
Lower bound (μg/kg)	56.2	21.1	58.9	88.3	47.1	47.5
Upper bound (μg/kg)	80.0	30.0	88.4	132	70.7	76.7

D.2.2. Pigs and poultry

Rapeseed meal may be fed at up to 5–10% for young pigs and to 15–20% for older pigs (McDonald et al., 2011).

Rapeseed meal may be included in diets for broilers and laying hens, at levels of about 30% and 20%, respectively (Canola Council of Canada, 2015), but is not recommended for young birds. Rapeseed oil may also be included in diets depending on its price relative to soybean and other vegetable oils. Some feed producers prefer rapeseed oil because of its lower linoleic acid content and also its non-genetically modified status, and rape oil will also potentially find its way into fat blends. In addition, whole (full-fat) rape seeds are now used as a key ingredient in broiler diets, at inclusion levels of up to 8%.

For some time, rapeseed meal was not recommended as a feed for laying hens, and particularly brown-shelled layers, because eggs tended to have a fishy flavour. It was found that these birds produced lower levels of trimethylamine oxidase than white leghorn type birds and since the trimethylamine was not metabolised it passed into the yolk, giving this taint. However, recent research has identified the gene responsible for this and breeding programmes have now removed the defect from most birds (Daun et al., 2011).

The assumed inclusion rates of rapeseed meal and rapeseed oil in diets of pigs and poultry, together with the calculated mean LB and UB concentrations of erucic acid, are given in Table D.4.

Table D.4: Assumed inclusion rates of rapeseed meal and rapeseed oil in the diets of pigs and poultry, and the calculated mean lower bound and upper bound concentrations of erucic acid in the total diet

Feeds	Piglets	Fattening pigs	Lactating sow	Broilers	Laying hens	Fattening turkeys	Fattening ducks
Rapeseed meal (%)	10	20	20	30	20	20	15
Rapeseed oil (%)	3	3	3	1.5	1.5	3	3
Estimated mean erucic acid concentration							
Lower bound (μg/kg)	85.4	131	131	157	111	131	108
Upper bound (μg/kg)	172	216	216	203	156	218	195

D.2.3. Farmed rabbits, fish (salmonids) and mink

Although soybean meal has been the protein supplement of choice for rabbit producers, rapeseed (Canola) meal may be used as a complete replacement (McNitt et al., 2013). Inclusion rates of 10% and 3% for rapeseed meal and rapeseed oil, respectively, have been used in estimating exposure to erucic acid (Table D.5).

As discussed elsewhere in this Opinion (Sections 3.3.1.6 and 3.3.5.5), there has been considerable research to identify optimum levels of rapeseed meal and/or oil in fish diets. In diets for aquatic species, the Canola Council of Canada recommends maximum inclusion levels of between 15% (for prawns) and 60% (Red Sea bream) (Canola Council of Canada, 2015). For salmon and trout, it recommends a maximum inclusion level of 20%, and this has been used to estimate exposure for a 2 kg salmon with a feed intake of 0.04 kg/day (EFSA FEEDAP Panel, 2012) (Table D.5).

In common with other plant proteins, rapeseed meals have relatively high levels of which mink cannot digest very well. As a result, commercially manufactured mink feed consists largely of fish and land animal by-products, with lesser amounts of cereals and cereal by-products, and supplemented with mineral/vitamin premixtures. The assumed inclusion rates of rapeseed meal and rapeseed oil in diets of mink together with the calculated mean lower bound and upper bound concentrations of erucic acid, are given in Table D.5.

Table D.5: Assumed inclusion rates of rapeseed meal and rapeseed oil in the diets of rabbits, fish (salmonids) and mink, and calculated mean lower bound (LB) and upper bound (UB) concentrations of erucic acid in the total diet

Feed materials	Rabbits	Farmed fish (Salmonids)	Farmed mink
Rapeseed meal	10	20	15
Rapeseed oil	3	5	3
Estimated mean erucic acid concentration			
Lower bound (μg/kg)	85.4	158	108
Upper bound (μg/kg)	172	302	195

Appendix E – Identification and selection of evidence relevant for the risk assessment of erucic acid in food and feed

E.1. Search for scientific literature

Chemistry and methods of analysis

A search in web of science and PubMed was conducted to identify papers on chemistry and methods of analysis by using the following search strings.

A. Web of science

Used search string: TOPIC: ("erucic acid" OR "*cis*-13-docosenoic acid" OR erucate) AND TOPIC: (chemistry OR analysis OR determination OR detection OR spectroscopy OR chromatography OR TLC OR GC OR GC-MS OR HPLC OR LC-MS OR ICP-MS) NOT TOPIC: (petroleum OR breeding OR biodiesel OR genome OR "genetic changes"); Refined by: RESEARCH AREAS: (AGRICULTURE OR FOOD SCIENCE TECHNOLOGY OR CHEMISTRY OR TOXICOLOGY); Timespan=1995-2015; Search language=Auto

Result in web of science: 399

B. PubMed

Used search string: (((((("erucic acid" OR "*cis*-13-docosenoic acid" OR erucate)) AND (chemistry OR analysis OR determination OR detection OR spectroscopy OR chromatography OR TLC OR GC OR GC-MS OR HPLC OR LC-MS OR ICP-MS)) NOT (petroleum OR breeding OR biodiesel OR genome OR "genetic changes"))) AND ("1995/1/1"[Date - Publication]: "3000"[Date - Publication])

Results in PubMed: 137

Occurrence, Exposure

A search in web of science and PubMed was conducted to identify papers on occurrence and exposure by using the following search strings.

A. Web of science

Used search string: TOPIC: ("erucic acid" OR "*cis*-13-docosenoic acid" OR erucate) AND TOPIC: (food OR "rapeseed oil" OR "mustard oil" OR "canola oil" OR brassica OR "fish oil" OR "vegetable oil" OR "dietary exposure" OR feed OR intake) NOT TOPIC: (petroleum OR breeding OR biodiesel OR genome OR "genetic changes"); Refined by: RESEARCH AREAS: (AGRICULTURE OR FOOD SCIENCE TECHNOLOGY OR CHEMISTRY OR FISHERIES OR VETERINARY SCIENCES); Timespan=1995-2015; Search language=Auto

Result in web of science: 751

B. PubMed

Used search string: ((((("erucic acid" OR "*cis*-13-docosenoic acid" OR educate)) AND (food OR "rapeseed oil" OR "mustard oil" OR "canola oil" OR brassica OR "fish oil" OR "vegetable oil" OR "dietary exposure" OR feed OR intake)) NOT (petroleum OR breeding OR biodiesel OR genome OR "genetic changes")) AND ("2015/1/1"[PDAT]: "3000"[PDAT])

Results in pubmed: 109

Human milk

A search in web of science and PubMed was conducted to identify papers on human milk by using the following search strings:

A. Web of science

TOPIC: ("human milk" OR "breast milk") *AND* **TOPIC:** ("fatty acid") *AND* **TOPIC:** (Europe); Timespan=1995-2016; Search language=Auto

Results in web of science: 131

B. PubMed

Used search string: Search (((("human milk" OR "breast milk")) *AND* **TOPIC:** ("fatty acid") *AND* **TOPIC:** (Europe) AND ("1995/01/01"[PDAT]: "3000"[PDAT])

Results in PubMed: 53

Processing

A search in web of science and PubMed was conducted to identify papers on processing by using the following search strings.

A. Web of science

Used search string: TOPIC: ("erucic acid" OR "*cis*-13-docosenoic acid" OR erucate) AND TOPIC: (cooking OR roasting OR frying OR boiling OR baking OR sterilization) NOT TOPIC: (petroleum OR breeding OR biodiesel OR genome OR "genetic changes"); Refined by: RESEARCH AREAS: (FOOD SCIENCE TECHNOLOGY OR AGRICULTURE OR CHEMISTRY); Timespan=All years; Search language=Auto

Result in web of science: 114

B. PubMed

Used search string: ((("erucic acid" OR "*cis*-13-docosenoic acid" OR erucate)) AND (cooking OR roasting OR frying OR boiling OR baking OR sterilization)) NOT (petroleum OR breeding OR biodiesel OR genome OR "genetic changes")

Results in PubMed: 6

Toxicity

A search in web of science and PubMed was conducted to identify papers on toxicity by using the following search strings.

A. Web of science

Used search string: TOPIC: ("erucic acid" OR "*cis*-13-docosenoic acid" OR erucate) AND TOPIC: (toxicity OR toxi* OR mutagen* OR teratogen* OR carcinogen* OR carcino* OR genotox* OR reprotox* OR nephrotox* OR neurotox* OR hepatotox* OR immunotox* OR haemotox* OR hematotox* OR cytotox* OR "develop* toxicity" OR endocri* OR "adverse effect" OR cardio* OR cardia*) NOT TOPIC: (petroleum OR breeding OR biodiesel); Refined by: RESEARCH AREAS: (RESPIRATORY SYSTEM OR CARDIOVASCULAR SYSTEM CARDIOLOGY OR HEMATOLOGY OR ENDOCRINOLOGY METABOLISM OR BIOCHEMISTRY MOLECULAR BIOLOGY OR ONCOLOGY OR TOXICOLOGY OR RHEUMATOLOGY OR CELL BIOLOGY OR PHARMACOLOGY PHARMACY OR GASTROENTEROLOGY HEPATOLOGY OR PHYSIOLOGY OR DEVELOPMENTAL BIOLOGY OR VETERINARY SCIENCES OR DERMATOLOGY OR NEUROSCIENCES NEUROLOGY OR ANATOMY MORPHOLOGY OR RESEARCH EXPERIMENTAL MEDICINE OR PATHOLOGY OR UROLOGY NEPHROLOGY OR REPRODUCTIVE BIOLOGY OR IMMUNOLOGY); Timespan=All years; Search language=Auto

Results in web of science: 561

B. PubMed

Used search string: ((("erucic acid" OR "*cis*-13-docosenoic acid" OR erucate)) AND (toxicity OR toxi* OR mutagen* OR teratogen* OR carcinogen* OR carcino* OR genotox* OR reprotox* OR nephrotox* OR neurotox* OR hepatotox* OR immunotox* OR haemotox* OR hematotox* OR cytotox* OR "develop* toxicity" OR endocri* OR "adverse effect" OR cardio* OR cardia*)

Results in PubMed: 115

Metabolism, kinetics

A search in web of science and PubMed was conducted to identify papers on metabolism and kinetics by using the following search strings.

A. Web of science

Used search string: TOPIC: ("erucic acid" OR "*cis*-13-docosenoic acid" OR erucate) AND TOPIC: (metabol* OR distribut* OR excret* OR absorp* OR biotransformation OR toxicokinetics) NOT TOPIC: (petroleum OR breeding OR biodiesel OR genome OR "genetic changes"); Refined by: RESEARCH AREAS: (BIOCHEMISTRY MOLECULAR BIOLOGY OR PHARMACOLOGY PHARMACY OR LIFE SCIENCES BIOMEDICINE OTHER TOPICS OR VETERINARY SCIENCES OR ENDOCRINOLOGY METABOLISM OR CARDIOVASCULAR SYSTEM CARDIOLOGY OR RESPIRATORY SYSTEM OR CELL BIOLOGY OR GASTROENTEROLOGY HEPATOLOGY OR PHYSIOLOGY OR TOXICOLOGY OR PUBLIC ENVIRONMENTAL OCCUPATIONAL HEALTH OR REPRODUCTIVE BIOLOGY OR RESEARCH EXPERIMENTAL MEDICINE OR UROLOGY NEPHROLOGY OR HEMATOLOGY); Timespan=All years; Search language=Auto; Timespan: all years

Result in web of science: 2,330

B. PubMed

Used search string: (((“erucic acid” OR “*cis*-13-docosenoic acid” OR erucate)) AND (metabol* OR distribut* OR excret* OR absorp* OR biotransformation OR toxicokinetics)) NOT (petroleum OR breeding OR biodiesel OR genome OR “genetic changes”)

Results in PubMed: 339

Human studies

A search in web of science and PubMed was conducted to identify papers on human data by using the following search strings.

A. Web of science

Used search string: TOPIC: (“erucic acid” OR “*cis*-13-docosenoic acid” OR erucate) AND TOPIC: (epidemiology OR biomarker OR cohort OR case control OR case stud* OR “incidental poisoning” OR “clinical stud*”) NOT TOPIC: (petroleum OR breeding OR biodiesel); Refined by: RESEARCH AREAS: (BIOCHEMISTRY MOLECULAR BIOLOGY OR REPRODUCTIVE BIOLOGY OR CARDIOVASCULAR SYSTEM CARDIOLOGY OR NEUROSCIENCES NEUROLOGY OR HEMATOLOGY OR ENDOCRINOLOGY METABOLISM OR DEVELOPMENTAL BIOLOGY OR TOXICOLOGY OR RESPIRATORY SYSTEM OR PEDIATRICS OR ONCOLOGY OR RHEUMATOLOGY OR PHYSIOLOGY OR GASTROENTEROLOGY HEPATOLOGY OR GERIATRICS GERONTOLOGY OR OBSTETRICS GYNECOLOGY OR PHARMACOLOGY PHARMACY OR ANATOMY MORPHOLOGY OR CELL BIOLOGY OR UROLOGY NEPHROLOGY OR IMMUNOLOGY OR PATHOLOGY OR PUBLIC ENVIRONMENTAL OCCUPATIONAL HEALTH); Timespan=All years; Search language=Auto

Result in web of science: 70

B. Pubmed

Used search string: (((“erucic acid” OR “*cis*-13-docosenoic acid” OR erucate)) AND (epidemiology OR biomarker OR cohort OR case control OR case stud* OR “incidental poisoning” OR “clinical stud*”)) NOT (petroleum OR breeding OR biodiesel)

Results in PubMed: 36

E.2. Exclusion criteria for abstracts

The references resulting from the literature search were imported and saved using a software package (EndNote[52]), which allows effective management of references and citations. After deletion of the duplicate reference, a list of about 1,900 references was obtained. The titles and abstracts of these references were screened to identify the relevant papers. Papers on the following subjects were excluded:

- Papers focused on nutritional aspects without studying toxic effects
- Papers related to plant science
- Papers on non-edible oil
- Papers related to antimicrobial/insecticide activity
- Papers related to quality of oil (e.g. oxidative stability, oil composition)
- Papers on environmental science
- Papers related to economic aspects
- Papers on compounds in oil other than erucic acid

E.3. EFSA guidance documents applied for the risk assessment

- EFSA (European Food Safety Authority), 2005. Opinion of the Scientific Committee on a request from EFSA related to a harmonised approach for risk assessment of substances which are both genotoxic and carcinogenic. EFSA Journal 2005;3(10):282, 31 pp. doi:10.2903/j.efsa.2005.282
- EFSA (European Food Safety Authority), 2006. Guidance of the Scientific Committee on a request from EFSA related to uncertainties in Dietary Exposure Assessment. EFSA Journal 2007;4(1):438, 54 pp. doi:10.2903/j.efsa.2007.438

[52] EndNote X5, Thomson Reuters. Available at: http://endnote.com/

- EFSA (European Food Safety Authority), 2009a. Guidance of the Scientific Committee on use of the benchmark dose approach in risk assessment. EFSA Journal 2009;6(6):1150, 72 pp. doi:10.2903/j.efsa.2009.1150
- EFSA (European Food Safety Authority), 2009b. Guidance of the Scientific Committee on transparency in the scientific aspects of risk assessments carried out by EFSA. Part 2: General principles. EFSA Journal 2009;6(5):1051, 22 pp. doi:10.2903/j.efsa.2009.1051
- EFSA (European Food Safety Authority), 2010a. Standard sample description for food and feed. EFSA Journal 2010;8(1):1457, 54 pp. doi:10.2903/j.efsa.2010.1457
- EFSA (European Food Safety Authority), 2010b. Management of left-censored data in dietary exposure assessment of chemical substances. EFSA Journal 2010;8(3):1557, 96 pp. doi:10.2903/j.efsa.2010.1557
- EFSA (European Food Safety Authority), 2011a. Guidance of EFSA on the use of the EFSA Comprehensive European Food Consumption Database in Intakes Assessment. EFSA Journal 2011;9(3):2097, 34 pp. doi:10.2903/j.efsa.2011.2097
- EFSA (European Food Safety Authority), 2011b. Overview of the procedures currently used at EFSA for the assessment of dietary exposure to different chemical substances. EFSA Journal 2011;9(12):2490, 33 pp. doi:10.2903/j.efsa.2011.2490
- EFSA Scientific Committee, 2012a. Guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual measured data. EFSA Journal 2012;10(3):2579, 32 pp. doi:10.2903/j.efsa.2012.2579
- EFSA Scientific Committee, 2012b. Scientific Opinion on Risk Assessment Terminology. EFSA Journal 2012;10(5):2664, 43 pp. doi:10.2903/j.efsa.2012.2664

Appendix F – Previous occurrence data

Table F.1: Erucic acid[(a)] concentration (mg/kg) in grains and grain-based products[(b)]

Product	N	Mean	Median	Range	Reference
Biscuits	17	29	0	0–200	Public Health England (2015)
Biscuits	8	0	0	0–0	US Department of Agriculture (2015)
Biscuits	12	2,792	0	0–14,600	National Food Institute of Denmark (2015)
Biscuits	27	237	0	0–3,660	Max Rubner Institut (2010)
Bread	74	2	0	0–40	US Department of Agriculture (2015)
Bread	33	6	0	0–200	Public Health England (2015)
Bread	12	0	0	0–0	NFI (2015)
Bread	258	160	20	0–1,500	Max Rubner Institut (2010)
Buñuelos	1	–	–	50	US Department of Agriculture (2015)
Cakes	32	2	0	0–30	US Department of Agriculture (2015)
Cakes	14	243	200	0–700	Public Health England (2015)
Cakes	6	10,412	11,450	320–14,700	NFI (2015)
Cakes	355	204	50	0–3,540	Max Rubner Institut (2010)
Cereal bars	2	50	50	0–100	Public Health England (2015)
Cereals ready-to-eat	145	1	0	0–50	US Department of Agriculture (2015)
Cereals ready-to-eat	27	48	0	0–500	Public Health England (2015)
Cereals ready-to-eat	1	–	–	0	NFI (2015)
Cereals ready-to-eat	70	1	0	0–10	Max Rubner Institut (2010)
Cookies	77	3	0	0–80	US Department of Agriculture (2015)
Cookies	2	410	410	0–820	NFI (2015)
Crackers	50	11	0	0–50	US Department of Agriculture (2015)
Crackers	1	–	–	0	Public Health England (2015)
Crackers	1	–	–	0	NFI (2015)
Crackers	3	937	820	820–1,170	Max Rubner Institut (2010)
Cream of wheat	11	1	0	0–10	US Department of Agriculture (2015)
Crispbreads	148	158	30	0–470	Max Rubner Institut (2010)
Croissants	6	5	0	0–30	US Department of Agriculture (2015)
Croissants	1	–	–	100	Public Health England (2015)
Croissants	1	–	–	530	NFI (2015)
Currant buns	1	–	–	100	Public Health England (2015)
Dough	8	33	30	0–100	Max Rubner Institut (2010)
Doughnuts	8	1	0	0–10	US Department of Agriculture (2015)
Doughnuts	2	50	50	0–100	Public Health England (2015)
Doughnuts	4	1,173	520	460–3,190	Max Rubner Institut (2010)
Dumplings	45	101	40	0–610	Max Rubner Institut (2010)
Flapjacks	1	–	–	200	Public Health England (2015)
Garlic bread	2	10	–	0–20	US Department of Agriculture (2015)
Muesli	1	–	–	100	Public Health England (2015)
Muffins	28	2	0	0–40	US Department of Agriculture (2015)
Muffins	3	567	800	0–900	Public Health England (2015)
Muffins	8	720	670	0–1,040	Max Rubner Institut (2010)
Oatcakes	1	–	–	100	Public Health England (2015)
Pancakes	40	147	30	0–1,960	Max Rubner Institut (2010)
Pasta	100	0	0	0–10	Max Rubner Institut (2010)
Pastries	13	0	0	0–0	US Department of Agriculture (2015)
Pastries	11	209	200	100–600	Public Health England (2015)
Pastries	13	0	0	0–0	NFI (2015)
Pastries	230	674	50	0–24,660	Max Rubner Institut (2010)

Product	N	Mean	Median	Range	Reference
Pie	36	0	0	0–0	US Department of Agriculture (2015)
Pie	6	133	100	0–300	Public Health England (2015)
Quinoa	3	1,008	1,004	954–1,066	Wood et al. (1993)
Quinoa	2	55	55	30–80	Max Rubner Institut (2010)
Rolls and buns	175	92	0	0–330	Max Rubner Institut (2010)
Scones	7	29	0	0–100	Public Health England (2015)
Semolina	2	0	0	0–0	US Department of Agriculture (2015)
Semolina	1	–	–	0	NFI (2015)
Semolina	5	36	5	0–160	Max Rubner Institut (2010)
Strudel	1	–	–	300	Public Health England (2015)
Tarts	3	200	200	100–300	Public Health England (2015)
Wafers	2	15	15	0–30	US Department of Agriculture (2015)
Wafers	1	–	–	100	Public Health England (2015)
Waffles	8	3	0	0–20	US Department of Agriculture (2015)
Waffles	20	944	115	0–3,960	Max Rubner Institut (2010)
Wheat rusk	2	585	585	510–660	NFI (2015)

(a): The database of the Max Rubner Institut reports 22:1, without differentiation of n-9 and n-11 isomers or *cis* and *trans*. US Department of Agriculture database reports the total 22:1*c* content, without differentiation of n-9 and n-11 isomers. The database of Public Health England reports separately 22:1 n-9*c*+*t* and 22:1 n-11 *c*+*t*. Only data of 22:1 n-9*c*+*t* is included in the table. The database of the National Food Institute of Denmark reports data on 22:1 n-9*c*.

(b): The following are products included in the nutrient databases of the US Department of Agriculture, Public Health England, National Food Institute of Denmark and Max Rubner Institut for which no erucic acid has been reported. The cumulative number of samples included in both databases is given in brackets: Amaranth (1); bagels (12); bannocks (2); barley (25); biscuits (4); breakfast bars (2); buckwheat (16); bulgur (3); cereals and cereals flour (16); cheesecake (5); coffeecake (6); corn (15); cous cous (3), cream of rice (3); croutons (2); crumble (9); farina (5); flan (11); gluten meat (4); malted drink mix (8); millet (16); oats (17); polenta (4); popcorn (3); rice (101); rye (31); sorghum flour (1); Spelt flour (12); Swiss rolls (1); turnovers (2); wheat (74).

Table F.2: Erucic acid[(a)] concentration (mg/kg) in vegetables and vegetable products[(b)]

Product	N	Mean	Median	Range	Reference
Broccoli sprouts	1	–	–	3,200	West et al. (2002)
Brassica oleracea sprouts	8	28,277	27,438	13,563–48,048	Vale et al. (2015)
Canola sprouts[(c)]	4	34,607	25,024	1,101–87,280	Bhardwaj and Hamama (2009)
Instant oatmeal	12	17	20	0–50	US Department of Agriculture (2015)
Kale leaves	1	–	–	2	Ayaz et al. (2006)
Pickled vegetables with mustard	2	550	550	460–640	Max Rubner Institut (2010)

(a): The database of the Max Rubner Institut reports 22:1, without differentiation of n-9 and n-11 isomers or *cis* and *trans*. US Department of Agriculture database reports the total 22:1*c* content, without differentiation of n-9 and n-11 isomers. The database of Public Health England reports separately 22:1 n-9*c*+*t* and 22:1 n-11 *c*+*t*. Only data of 22:1 n-9*c*+*t* is included in the table. The database of the National Food Institute of Denmark reports data on 22:1 n-9*c*.

(b): The following are products included in the nutrient databases of the US Department of Agriculture, Public Health England, National Food Institute of Denmark and Max Rubner Institut for which no erucic acid has been reported. The cumulative number of samples included in both databases is given in brackets: arrowhead (2); alfalfa sprouts (6); amaranth leaves (2); artichoke (23); asparagus (28); aubergine (30); bamboo shoots (16); balsam pear (2); beetroot (31); beet greens (6); betel leaves (1); borage (5); broccoli (42); Brussels sprouts (25); bush beans (8); cabbage (86); capers (1); cardoon (7); carrots (42); cauliflower (24); celeriac (24); celery (16); chard (14); chayote (11); chicory (10); chicory greens (2); Chinese cabbage (13); cocoa powder (17); coconut (1); collards (5); coriander leaves (1); courgettes (8); cress (8); cucumber (20); dandelion greens (10); drumstick, leaves and pods (4); endive (9); escarole (1); fennel (17); fungi (100); garlic (27); gherkins, raw and pickled (2); gourd (7); grape leaves (4); horseradish (10); instant breakfast powder (13); instant chocolate (21); instant coffee (16); kale (23); kohlrabi (23); leek (21); lettuce (27); marrow (3); morel (11); mushrooms (57); nopal (2); okra (20); onions (80); orache (8); pak choi (2); palm heart (7); parsley (7); parsnip (16); peppermint (2); peppers (140); pickled vegetables (18); popcorn (5); pumpkin (44); purslane (11); radicchio (6); radish (33); radish leaves (1); rape leaves (1); rhubarb (18); Romanesco (2); root parley (13); rutabaga (3); salad rocket (2); salsify (16); sauerkraut (20); seaweeds (26); shallots (8); sorrel (6); spinach (41); squash (32); stinging nettle (8); swede (2); sweet corn (25); tomatoes (74); tea (11); truffle (10); turnip (34); turnip greens (12); vegetables mix (118); watercress (9); water chestnut (5); Welsh onion (10); zucchini (25).

(c): The authors reported the use of four canola cultivars although two of them should be considered as rapeseed instead of canola according to the definition provided in Section 1.3.1 of this opinion.

Table F.3: Erucic acid[(a)] concentration (mg/kg) in starchy roots and tubers[(b)]

Product	N	Mean	Median	Range	Reference
French fries	8	21	20	0–40	US Department of Agriculture (2015)
French fries	1	–	–	110	NFI (2015)
French fries	7	1,169	0	0–4,290	Max Rubner Institut (2010)
Sweet potato	11	4	0	0–40	US Department of Agriculture (2015)
Sweet potato	1	–	–	0	NFI (2015)
Sweet potato	5	0	0	0–0	Max Rubner Institut (2010)

(a): The database of the Max Rubner Institut reports 22:1, without differentiation of n-9 and n-11 isomers or *cis* and *trans*. US Department of Agriculture database reports the total 22:1*c* content, without differentiation of n-9 and n-11 isomers. The database of Public Health England reports separately 22:1 n-9*c*+*t* and 22:1 n-11 *c*+*t*. Only data of 22:1 n-9*c*+*t* is included in the table. The database of the National Food Institute of Denmark reports data on 22:1 n-9*c*.

(b): The following are products included in the nutrient databases of the US Department of Agriculture, Public Health England, National Food Institute of Denmark and Max Rubner Institut for which no erucic acid has been reported. The cumulative number of samples included in both databases is given in brackets: arrowroot (8); burdock root (2); cassava (10); ginger (3); Jerusalem artichoke (7); lotus root (2); potato (82); tapioca (2); taro (13); yucca chips (1); yam (14); yam bean (6).

Table F.4: Erucic acid[(a)] concentration (mg/kg) in legumes, nuts and oilseeds[(b)]

Product[(d),(e)]	N	Mean	Median	Range	Reference
Black mustard	5	143,352	137,120	122,653–192,921	Tahoun et al. (1999)
Borage	6	12,794	7,236	3,060–42,744	de Haro et al. (2002)
Camelina	8	12,646	12,530	11,360–13,870	Budin et al. (1995)
Canola	3[(c)]	44	44	43–45	Barthet (2014)
Cowpea	4	29	0	0–117	Antova et al. (2014)
Crambe	1	–	–	191,310	Lalas et al. (2012)
Ethiopian mustard	5	148,111	144,146	79,072–198,852	Tahoun et al. (1999)
Hazelnut	3	0	0	0–0	US Department of Agriculture (2015)
Hazelnut	1	–	–	0	Public Health England (2015)
Hazelnut	10	55	0	0–280	Max Rubner Institut (2010)
Hazelnut	1	–	–	0	NFI (2015)
Kola nut	3	203	300	0–310	Max Rubner Institut (2010)
Lupines	2	1,928	1,928	1,699–2,157	Bhardwaj et al. (2004)
Lupines	6	1,839	1,841	1,206–2,457	Oliveira and Ferreira (1988)
Lupines	18	2,221	2,062	815–4,942	Boschin et al. (2008)
Macadamia	4	1,185	1,185	0–2,370	Max Rubner Institut (2010)
Mustard seeds	2	115,630	115,630	115,630–115,630	Max Rubner Institut (2010)
Nuts	8	143	130	0–270	US Department of Agriculture (2015)
Nuts	3	307	460	0–460	Max Rubner Institut (2010)
Oriental mustard	5	115,605	106,240	69,758–178,144	Tahoun et al. (1999)
Peanuts	8	135	0	0–540	US Department of Agriculture (2015)
Peanuts	9	247	320	0–480	Max Rubner Institut (2010)
Rape seed	4	103,646	103,809	51,935–155,030	Tahoun et al. (1999)
Sesame	3	0	0	0–0	US Department of Agriculture (2015)
Sesame	1	–	–	0	Public Health England (2015)
Sesame	1	–	–	0	NFI (2015)
Sesame	6	92	0	0–280	Max Rubner Institut (2010)
Sunflower	7	0	0	0–0	US Department of Agriculture (2015)
Sunflower	2	0	0	0–0	Public Health England (2015)
Sunflower	8	72	0	0–280	Max Rubner Institut (2010)
Turnip rape	5	136,426	140,624	70,686–177,390	Tahoun et al. (1999)
Walnuts	3	0	0	0–0	US Department of Agriculture (2015)
Walnuts	1	–	–	0	Public Health England (2015)

Product[(d),(e)]	N	Mean	Median	Range	Reference
Walnuts	1	–	–	0	NFI (2015)
Walnuts	17	32	0	0–280	Max Rubner Institut (2010)

(a): The database of the Max Rubner Institut reports 22:1, without differentiation of n-9 and n-11 isomers or *cis* and *trans*. US Department of Agriculture database reports the total 22:1*c* content, without differentiation of n-9 and n-11 isomers. The database of Public Health England reports separately 22:1 n-9*c*+*t* and 22:1 n-11 *c*+*t*. Only data of 22:1 n-9*c*+*t* is included in the table. The database of the National Food Institute of Denmark reports data on 22:1 n-9*c*.

(b): The following are products included in the nutrient databases of the US Department of Agriculture, Public Health England, National Food Institute of Denmark and Max Rubner Institut for which no erucic acid has been reported. The cumulative number of samples included in both databases is given in brackets: almond (30); beans (167); bean sprouts (14); beech nut (4); betel nut (1); black gram (6); Brazil nut (10); breadnut (1); broad beans (2); butternut (1); carob flour (1); cashew (19); chestnuts (21); chevra (1); chickpea (25); chickpea sprouts (1); coconut (10); cottonseed (2)[d]; fenugreek (2); ginkgo nut (1); hickorynut (6); lablab (4); lentil (37); lentil sprouts (2); lima bean (11); linseed (11); mungbean (21); mungbean sprouts (2); peas (86); pea sprouts (2); pecans (12); pigeon pea (10); pine nuts (6); pistachio (8); poppy seed (4); pumpkin seed (14); safflower seeds (0)[e]; soy flour (6); soybeans (31)

(c): Refers to number of years (2010, 2011, 2012) of harvest surveys in Western Canada, including 2,108, 1,749 and 1,641 samples/year, respectively.

(d): Five samples of cottonseed seeds and meal with 22:1 content between 30 and 370 mg/kg (Max Rubner Institut, 2010) are not included because there is scientific evidence that cotton seeds lack 22:1 fatty acids (Dowd et al., 2010).

(e): Two samples of safflower seeds with 22:1 content of 370 mg/kg each (Max Rubner Institut, 2010) are not included because there is scientific evidence that safflower seeds lack 22:1 fatty acids (Matthaus et al., 2015).

Table F.5: Erucic acid[(a)] concentration (mg/kg) in fruit and fruit products[(b)]

Product	N	Mean	Median	Range	Reference
Plantains	4	3	0	0–10	US Department of Agriculture (2015)
Plantains	6	0	0	0–0	Max Rubner Institut (2010)

(a): The database of the Max Rubner Institut reports 22:1, without differentiation of n-9 and n-11 isomers or *cis* and *trans*. US Department of Agriculture database reports the total 22:1*c* content, without differentiation of n-9 and n-11 isomers. The database of Public Health England reports separately 22:1 n-9*c*+*t* and 22:1 n-11 *c*+*t*. Only data of 22:1 n-9*c*+*t* is included in the table. The database of the National Food Institute of Denmark reports data on 22:1 n-9*c*.

(b): The following are products included in the nutrient databases of the US Department of Agriculture, Public Health England, National Food Institute of Denmark and Max Rubner Institut for which no erucic acid has been reported. The cumulative number of samples included in both databases is given in brackets: acerola (10); ackee (9); amla (1); apple (55); apple butter (2); apricot (33); avocado (15); babaco (1); banana (23); bilberry(3); blackberry (20); blackcurrant (3); blueberry (10); boysenberry (9); breadfruit (9); Cape gooseberry (7); carambola (1); Carissa (7); cashew apple (9); chayote (3); cherimoya (24); cherries (60); coconut (3); cranberry (20); currants (34); damsons (6); dates (15); durian (9); elderberry (8); feijoa (5); fig (21); fruit compote (57); fruit mix (22); fruit paste (2); fruit salad (8); gooseberry (15); grapefruit (55); greengages (6); grenadilla (7); grüne Grütze (3); guava (20); hackelberry (2); jabuticaba (6); jackfruit (9); jam (121); jujube (8); kaki (10); kiwi (14); kumquat (9); lemon (12); lime (9); litchi (13); loganberry (12); longan (10); loquats (2); lychees (4); mamey (9); mandarine (39); mango (13); mangosteen (7); medlar (12); melon (44); mulberry (10); naranjilla (9); nectarine (13); olive (16); orange (16), orange peel (1); papaya (15); passion fruit (12); peach (35); pear (37); persimmon (1); physalis (1); pineapple (39); plum (91); pokeberry (1); pomelo (2); pomegranate (10); prickly pear (7); prunes (9); quince (14); raisin (8); rambutan (2); raspberry (25); redcurrant (3); rose-apple (7); rosehip (9); röte Grütze (9); rowan (7); sapote (9); sapotilla (9); seabuckhorn (9); sloe (3); strawberry (26); Surinam cherry (7); tamarillo (8); tamarind (4); tangerines (5); tree gooseberry (9); watermelon (4); white currant (3); wild gooseberry (6); wild fruits (2).

Table F.6: Erucic acid[(a)] concentration (mg/kg) in meat and meat products[(b)]

Product	N	Mean	Median	Range	Reference
Bacon	12	17	0	0–60	US Department of Agriculture (2015)
Bacon	6	0	0	0–0	Public Health England (2015)
Bacon	3	0	0	0–0	NFI (2015)
Bacon	21	0	0	0–0	Max Rubner Institut (2010)
Beef, raw	339	0	0	0–0	US Department of Agriculture (2015)
Beef, raw	8	0	0	0–0	NFI (2015)
Beef, raw	273	0	0	0–0	Max Rubner Institut (2010)
Beef, preserve	6	0	0	0–0	Max Rubner Institut (2010)
Beef	14	14	0	0–200	Public Health England (2015)
Bologna	10	176	0	0–890	US Department of Agriculture (2015)

Product	N	Mean	Median	Range	Reference
Chicken, raw	56	3	0	0–50	US Department of Agriculture (2015)
Chicken, raw	10	0	0	0–0	NFI (2015)
Chicken, raw	23	3	0	0–60	Max Rubner Institut (2010)
Chicken	16	100	0	0–500	Public Health England (2015)
Corn dogs	1	–	–	280	US Department of Agriculture (2015)
Faggots	1	–	–	100	Public Health England (2015)
Frankfurter	13	136	0	0–1,180	US Department of Agriculture (2015)
Frankfurter	1	–	–	0	Public Health England (2015)
Lamb, raw	55	7	0	0–340	US Department of Agriculture (2015)
Lamb, raw	5	0	0	0–0	NFI (2015)
Lamb, raw	151	0	0	0–0	Max Rubner Institut (2010)
Lamb	14	0	0	0–0	Public Health England (2015)
Mince pie	2	100	100	0–200	Public Health England (2015)
Offal	130	30	0	0–450	Max Rubner Institut (2010)
Pâté	5	0	0	0–0	US Department of Agriculture (2015)
Pâté	17	55	20	0–290	Max Rubner Institut (2010)
Pâté	2	0	0	0–0	Public Health England (2015)
Pepperoni	1	–	–	40	US Department of Agriculture (2015)
Pigeon	12	66	70	50–80	Max Rubner Institut (2010)
Pork, raw	73	0	0	0–10	US Department of Agriculture (2015)
Pork, raw	6	0	0	0–0	NFI (2015)
Pork, raw	359	0	0	0–0	Max Rubner Institut (2010)
Pork, preserve	8	0	0	0–0	Max Rubner Institut (2010)
Pork	22	23	0	0–200	Public Health England (2015)
Salami	6	38	0	0–230	US Department of Agriculture (2015)
Salami	2	0	0	0–0	Public Health England (2015)
Sausages	46	60	0	0–930	US Department of Agriculture (2015)
Sausages	17	6	0	0–100	Public Health England (2015)
Sausages	10	0	0	0–0	NFI (2015)
Sausages	293	35	0	0–630	Max Rubner Institut (2010)
Soy sausages	3	3,527	4,020	2,540–4,020	Max Rubner Institut (2010)
Terrine	2	5	5	0–10	Max Rubner Institut (2010)
Turkey, raw	43	10	10	0–50	US Department of Agriculture (2015)
Turkey, raw	24	68	105	10–120	Max Rubner Institut (2010)
Turkey	4	150	100	100–300	Public Health England (2015)
Turkey	1	–	–	0	NFI (2015)
Veal, raw	16	0	0	0–0	US Department of Agriculture (2015)
Veal, raw	269	0	0	0–10	Max Rubner Institut (2010)
Veal, preserve	5	10	10	0–20	Max Rubner Institut (2010)
Veal	2	0	0	0–0	Public Health England (2015)
Veal	1	–	–	0	NFI (2015)

(a): The database of the Max Rubner Institut reports 22:1, without differentiation of n-9 and n-11 isomers or *cis* and *trans*. US Department of Agriculture database reports the total 22:1*c* content, without differentiation of n-9 and n-11 isomers. The database of Public Health England reports separately 22:1 n-9*c*+*t* and 22:1 n-11 *c*+*t*. Only data of 22:1 n-9*c*+*t* is included in the table. The database of the National Food Institute of Denmark reports data on 22:1 n-9*c*.

(b): The following are products included in the nutrient databases of the US Department of Agriculture, Public Health England, National Food Institute of Denmark and Max Rubner Institut for which no erucic acid has been reported. The cumulative number of samples included in both databases is given in brackets: animal fat (8); bison (2); caribou (1); chital (1); cured meat (7); deer (32); duck (65); emu (5); game (21); goat (8); goose (41); Guinea fowl (2); grouse (2); ham (116); hare (2); horse (9); kassler (28); luncheon (6); meat mix (35); mincemeat (2); mortadella (1); mutton (2); ostrich (2); ox (12); partridge (4); pheasant (13); poularde (4); quail (8); rabbit (39); reindeer (8); squab (4); textured soy protein (10); venison (3); wild boar (10).

Table F.7: Erucic acid[(a)] concentration (mg/kg) in fish and other seafood[(b)]

Product	N	Mean	Median	Range	Reference
Black Sea horse mackerel	21	54		Not reported	Stancheva et al. (2012)
Catfish	2	295	295	0–590	NFI (2015)
Cod	7	157	0	0–500	Public Health England (2015)
Cod	6	0	0	0–0	NFI (2015)
Crab	2	100	100	0–200	Public Health England (2015)
Crab	3	840	570	570–1,380	NFI (2015)
Finfish	37	170	30	0–1,680	Ackman (2008)
Fish	1			300	Public Health England (2015)
Fish, fried	3	100	100	0–200	NFI (2015)
Fish pâtés	7	2,200	1,900	400–4,300	Aquerreta et al. (2002)
Halibut	5	1,660	0	0–4,300	NFI (2015)
Herring	1	–	–	0	Public Health England (2015)
Herring	4	1,393	1,425	240–2,480	NFI (2015)
Lemon sole	3	0	0	0–0	Public Health England (2015)
Lemon sole	1	–	–	120	NFI (2015)
Lobster	4	470	465	440–510	NFI (2015)
Mackerel	1	–	–	2,100	Public Health England (2015)
Mackerel	4	1,505	1,440	950–2,190	NFI (2015)
Mussels	2	0	0	0–0	Public Health England (2015)
Mussels	4	718	480	0–1,910	NFI (2015)
Oyster	2	0	0	0–0	Public Health England (2015)
Oyster	1	–	–	340	NFI (2015)
Plaice	2	150	150	0–300	Public Health England (2015)
Pollock	2	50	50	0–100	Public Health England (2015)
Redfish	1	–	–	640	NFI (2015)
Roe	2	200	200	100–300	Public Health England (2015)
Round goby	12	320		Not reported	Stancheva et al. (2012)
Salmon	13	833	600	200–1,800	Public Health England (2015)
Salmon	7	1,139	800	0–3,990	NFI (2015)
Sardines	3	300	300	300–300	Public Health England (2015)
Sardines	3	0	0	0–0	NFI (2015)
Sea bass	2	250	250	200–300	Public Health England (2015)
Scampi	1			600	Public Health England (2015)
Shad	6	4,077		Not reported	Stancheva et al. (2012)
Sole	1	–	–	120	NFI (2015)
Sprat	1			600	Public Health England (2015)
Sprat	33	1,003		Not reported	Stancheva et al. (2012)
Sprat	1	–	–	2,390	NFI (2015)
Torsk	1	–	–	30	NFI (2015)
Trout	2	100	100	100–100	Public Health England (2015)
Trout	1	–	–	520	NFI (2015)
Tuna	5	20	0	0–100	Public Health England (2015)
Tuna	2	0	0	0–0	NFI (2015)

Product	N	Mean	Median	Range	Reference
Wolfish	1	–	–	320	NFI (2015)
Whelks	1	–	–	200	Public Health England (2015)

(a): The database of Public Health England reports separately 22:1 n-9 *c*+*t* and 22:1 n-11 *c*+*t*. Only data of 22:1 n-9*c*+*t* is included in the table. The database of the National Food Institute of Denmark reports data on 22:1 n-9*c*.

(b): The following are products included in the nutrient databases of Public Health England and National Food Institute of Denmark for which no erucic acid has been reported. The number of samples included in the database is given in brackets: anchovy (1); bucklingpaté (1); calamari (1); carp (1); caviar (2); cod liver oil (1); coley (6); crayfish (1); cuttlefish (1); dogfish (9); eel (4); fish fingers (1); flounder (4); flying fish (1); garfish (1); haddock (26); hake (3); halibut (5); hare (1); hoki (2); jackfish (1); langoustine (1); lemon sole (5); monkfish (3); mullet (6); octopus (1); parrot fish (1); plaice (2); prawns (12); red snapper (5); sea bream (1); seaweed (4); shark (1); shrimps (8); skate (3); snail (1); squid (2); stockfish (1); swordfish (3); turbot (4); whiting (9).

Table F.8: Erucic acid concentration (mg/kg) in milk and dairy products[(a),(b)]

Product	N	Mean	Median	Range	Reference
Cheese	95	3	0	0–80	US Department of Agriculture (2015)
Cheese	19	32	0	0–400	Public Health England (2015)
Cheese	3	0	0	0–0	NFI (2015)
Cheese	390	1	0	0–40	Max Rubner Institut (2010)
Cream	8	6	0	0–30	US Department of Agriculture (2015)
Cream	3	0	0	0–0	Public Health England (2015)
Cream	3	0	0	0–0	NFI (2015)
Cream	44	11	0	0–230	Max Rubner Institut (2010)
Milk	32	0	0	0–0	US Department of Agriculture (2015)
Milk	13	15	0	0–100	Public Health England (2015)
Milk	5	0	0	0–0	NFI (2015)
Milk	50	3	0	0–10	Max Rubner Institut (2010)
Milk-based beverages	6	0	0	0–0	US Department of Agriculture (2015)
Milk-based beverages	81	4	0	0–10	Max Rubner Institut (2010)
Yogurt	16	0	0	0–0	US Department of Agriculture (2015)
Yogurt	8	0	0	0–0	Public Health England (2015)
Yogurt	187	0	0	0–10	Max Rubner Institut (2010)

(a): The database of the Max Rubner Institut reports 22:1, without differentiation of n-9 and n-11 isomers or *cis* and *trans*. US Department of Agriculture database reports the total 22:1*c* content, without differentiation of n-9 and n-11 isomers. The database of Public Health England reports separately 22:1 n-9*c*+*t* and 22:1 n-11 *c*+*t*. Only data of 22:1 n-9*c*+*t* is included in the table. The database of the National Food Institute of Denmark reports data on 22:1 n-9*c*.

(b): The following are products included in the nutrient databases of the US Department of Agriculture, Public Health England, National Food Institute of Denmark and Max Rubner Institut for which no erucic acid has been reported. The cumulative number of samples included in both databases is given in brackets: buttermilk (15); cheese imitation (11); evaporated/ condensed milk (14); crème caramel (2); crème fraîche (2); cream substitute (1); kefir (23); milk powder (8); quark (19); sour cream imitation (1); soy yogurt (2); soy drink (9); tofu (26); whey (17); ymer (1).

Table F.9: Erucic acid content (mg/kg) in sugar and confectionary[(a),(b)]

Product	N	Mean	Median	Range	Reference
Chocolate	6	0	0	0–0	US Department of Agriculture (2015)
Chocolate	11	82	0	0–900	Public Health England (2015)
Chocolate	4	0	0	0–0	NFI (2015)
Chocolate	156	1	0	0–100	Max Rubner Institut (2010)
Chocolate bar	1	–	–	300	Public Health England (2015)
Chocolate bar	19	92	0	0–910	Max Rubner Institut (2010)

Product	N	Mean	Median	Range	Reference
Dragees	9	41	0	0–370	Max Rubner Institut (2010)
Fondant	11	4	0	0–20	Max Rubner Institut (2010)

(a): The database of the Max Rubner Institut reports 22:1, without differentiation of n-9 and n-11 isomers or *cis* and *trans*. US Department of Agriculture database reports the total 22:1*c* content, without differentiation of n-9 and n-11 isomers. The database of Public Health England reports separately 22:1 n-9*c*+*t* and 22:1 n-11 *c*+*t*. Only data of 22:1 n-9*c*+*t* is included in the table. The database of the National Food Institute of Denmark reports data on 22:1 n-9*c*.

(b): The following are products included in the nutrient databases of the US Department of Agriculture, Public Health England, National Food Institute of Denmark and Max Rubner Institut for which no erucic acid has been reported. The cumulative number of samples included in both databases is given in brackets: brandy snaps (1); brownies (1); candies (93); chewing gum (2); chocolate syrup (3); chocolate buns (1); dessert toping powder (4); frostings (9); fructose (1); fruit gums (1); gateau (2); glucose (1); honey (8); invert sugar (1); jelly (13); lactose (1); maltose (1); marshmallow (1); marzipan (9); nougat (4); sugar (14); sugared fruits (22); sweets (1); sweetener (19); syrup (25); toppings (5).

Table F.10: Erucic acid[(a)] concentration (mg/kg) in animal and vegetable fats and oils[(b)]

Product	N	Mean	Median	Range	Reference
Butter	7	7	0	0–50	US Department of Agriculture (2015)
Butter	4	500	400	0–1,200	Public Health England (2015)
Butter	1	–	–	0	NFI (2015)
Butter	27	1,080	420	0–9,560	Max Rubner Institut (2010)
Cooking fat	1	–	–	56,960	Max Rubner Institut (2010)
Cooking oil	1	–	–	1,410	US Department of Agriculture (2015)
Corn oil	1	–	–	0	US Department of Agriculture (2015)
Corn oil	1	–	–	0	NFI (2015)
Corn oil	1	–	–	1,910	Max Rubner Institut (2010)
Cottonseed oil	2	0	0	0–0	US Department of Agriculture (2015)
Cottonseed oil	1	–	–	0	Public Health England (2015)
Cottonseed oil	1	–	–	0	NFI (2015)
Cottonseed oil	1	–	–	1,910	Max Rubner Institut (2010)
Fat spreads	9	1,267	1,200	0–2,600	Public Health England (2015)
Flaxseed oil	2	175	175	170–180	US Department of Agriculture (2015)
Margarine	39	39	0	0–330	US Department of Agriculture (2015)
Margarine	1	–	–	2,200	Public Health England (2015)
Margarine	10	0	0	0–0	NFI (2015)
Margarine	23	21,071	17,210	2,680–45,890	Max Rubner Institut (2010)
Margarine	12	6,087	2,492	0–21,978	Kohiyama et al. (1990)
Mustard oil	5	113,540	47,600	9,100–341,200	Chowdhury et al. (2007)
Mustard oil	2	308,950	308,950	239,000–378,900	Abul-Fadl et al. (2011)
Mustard oil	3	385,030	418,000	217,300–519,800	Sarwar et al. (2014)
Mustard oil	9	248,330	215,000	3,000–508,000	Wendlinger et al. (2014)
Palm oil	2	0	0	0–0	US Department of Agriculture (2015)
Palm oil	1	–	–	0	Public Health England (2015)
Palm oil	1	–	–	0	NFI (2015)
Palm oil	2	1,910	1,910	1,910–1,910	Max Rubner Institut (2010)
Palm kernel oil	6	0	0	0–0	US Department of Agriculture (2015)
Palm kernel oil	1	–	–	0	NFI (2015)
Palm kernel oil	1	–	–	940	Max Rubner Institut (2010)
Peanut butter	10	197	0	0–1,090	US Department of Agriculture (2015)
Peanut butter	1	0		0	Public Health England (2015)
Peanut butter	5	274	440	0–470	Max Rubner Institut (2010)
Peanut oil	1	–	–	0	US Department of Agriculture (2015)
Peanut oil	1	–	–	1,000	Public Health England (2015)
Peanut oil	1	–	–	0	NFI (2015)

Product	N	Mean	Median	Range	Reference
Peanut oil	1	–	–	960	Max Rubner Institut (2010)
Rapeseed oil	1	–	–	2,000	Public Health England (2015)
Rapeseed oil	1	–	–	5,080	NFI (2015)
Rapeseed oil	1	–	–	2,990	Max Rubner Institut (2010)
Rapeseed oil from China	2	244,500	244,500	92,000–397,000	Wallingford et al. (2004)
Safflower oil	2	0	0	0–0	US Department of Agriculture (2015)
Safflower oil	1	–	–	0	Public Health England (2015)
Safflower oil	1	–	–	0	NFI (2015)
Safflower oil	1	–	–	960	Max Rubner Institut (2010)
Sesame oil	1	–	–	0	US Department of Agriculture (2015)
Sesame oil	1	–	–	0	Public Health England (2015)
Sesame oil	1	–	–	0	NFI (2015)
Sesame oil	1	–	–	0	Max Rubner Institut (2010)
Sesame oil from China	2	91,500	91,500	4,000–179,000	Wallingford et al. (2004)
Shortenings	16	0	0	0–0	US Department of Agriculture (2015)
Shortenings	3	57,897	58,740	56,210–58,740	Max Rubner Institut (2010)
Spread	1	–	–	190	NFI (2015)
Sunflower oil	1	–	–	1,000	Public Health England (2015)
Sunflower oil	1	–	–	0	NFI (2015)
Sunflower oil	2	475	475	0–950	Max Rubner Institut (2010)
Sunflower seed butter	2	30	30	30–30	US Department of Agriculture (2015)
Sunflower seed butter	1	–	–	460	Max Rubner Institut (2010)
Vegetable oil	1	–	–	2,000	Public Health England (2015)
Vegetable oil	3	0	0	0–0	Max Rubner Institut (2010)
Vegetable oil, pickles	71	22,110	3,000	0–270,000	Food Standards Agency (2004)
Vegetarian fat	3	15,320	16,440	13,080–16,440	Max Rubner Institut (2010)
Wheat germ oil	1	–	–	0	US Department of Agriculture (2015)
Wheat germ oil	1	–	–	0	Public Health England (2015)
Wheat germ oil	1	–	–	0	NFI (2015)
Wheat germ oil	1	–	–	1,900	Max Rubner Institut (2010)

(a): The database of the Max Rubner Institut reports 22:1, without differentiation of n-9 and n-11 isomers or *cis* and *trans*. US Department of Agriculture database reports the total 22:1*c* content, without differentiation of n-9 and n-11 isomers. The database of Public Health England reports separately 22:1 n-9*c*+*t* and 22:1 n-11 *c*+*t*. Only data of 22:1 n-9*c*+*t* is included in the table. The database of the National Food Institute of Denmark reports data on 22:1 n-9*c*.

(b): The following are products included in the nutrient databases of the US Department of Agriculture, Public Health England, National Food Institute of Denmark and Max Rubner Institut for which no erucic acid has been reported. The cumulative number of samples included in both databases is given in brackets: almond butter (1); almond oil (2); animal fat (1); apricot kernel oil (1); babassu oil (1); beef tallow (2); breadnut tree seed oil (1); chicken fat (1); cocoa butter (3); coconut oil (8); cupuassu oil (1); duck fat (1); evening primrose oil (1); frying oil (1); goose fat (2); grapeseed oil (4); hazelnut butter (3); hazelnut oil (3); kolanut butter (1); lard (3); linseed oil (2); mutton fat (1); nut butter (1); nutmeg butter (1); olive oil (4); poppy seed oil (2); pumpkin seed oil (1); rice bran oil (1); sesame butter (3); sesame oil (3); sheanut butter (2); shortenings (16); soybean oil (17); tea seed oil (1); tomato seed oil (1); ucuhuba butter (1); tallow (1); vegetable oil (3); walnut oil (4).

Table F.11: Erucic acid[(a)] content (mg/kg) in fruit and vegetable juices[(b)]

Product	N	Mean	Median	Range	Reference
Horchata	1			20	US Department of Agriculture (2015)

(a): The database of the Max Rubner Institut reports 22:1, without differentiation of n-9 and n-11 isomers or *cis* and *trans*. US Department of Agriculture database reports the total 22:1*c* content, without differentiation of n-9 and n-11 isomers. The database of Public Health England reports separately 22:1 n-9*c*+*t* and 22:1 n-11 *c*+*t*. Only data of 22:1 n-9*c*+*t* is included in the table. The database of the National Food Institute of Denmark reports data on 22:1 n-9*c*.

(b): The following are products included in the nutrient databases of the US Department of Agriculture, Public Health England, National Food Institute of Denmark and Max Rubner Institut for which no erucic acid has been reported. The cumulative number of samples included in both databases is given in brackets: açai berry drink (1); acerola juice (1); almond milk (3); Aloe vera juice (1); apple juice (9); apple sauce (2); apricot juice (1); barley water (4); blackberry juice (1); blackcurrant juice (2); carrot juice (1); cereal grain beverage (3); citrus fruit juice (2); cocoa butter (1); coconut milk (7); cranberry juice (6); fruit juice (217); fruit juice drink (35); grapefruit juice (14); lemon juice (6); lemonade (11); lime juice (5); orange juice (16); passion fruit juice (3); pear nectar (1); pineapple juice (6); pomelo juice (1); pomegranate juice (3); prune juice (2); rice milk (1); tangerine juice (2); tomato juice (5); tomato sauce (3); vegetable juice (8).

Table F.12: Erucic acid[(a)] content (mg/kg) in non-alcoholic beverages (excepting milk-based beverages)[(b)]

Product	N	Mean	Median	Range	Reference
Cocoa drink	2	1,915	1,915	380–3,450	Max Rubner Institut (2010)
Coffee	8	0	0	0–0	US Department of Agriculture (2015)
Coffee	1	–	–	0	Public Health England (2015)
Coffee	39	1	0	0–10	Max Rubner Institut (2010)

(a): The database of the Max Rubner Institut reports 22:1, without differentiation of n-9 and n-11 isomers or *cis* and *trans*. US Department of Agriculture database reports the total 22:1*c* content, without differentiation of n-9 and n-11 isomers. The database of Public Health England reports separately 22:1 n-9*c*+*t* and 22:1 n-11 *c*+*t*. Only data of 22:1 n-9*c*+*t* is included in the table. The database of the National Food Institute of Denmark reports data on 22:1 n-9*c*.

(b): The following are products included in the nutrient databases of the US Department of Agriculture, Public Health England, National Food Institute of Denmark and Max Rubner Institut for which no erucic acid has been reported. The cumulative number of samples included in both databases is given in brackets: Soft drink (72); other non-alcoholic beverages (14); tea (57).

Table F.13: Erucic acid[(a)] concentration (mg/kg) in herbs, spices and condiments[(b)]

Product	N	Mean	Median	Range	Reference
Chutney	3	23	20	20–30	Max Rubner Institut (2010)
Cloves	1	–	–	180	US Department of Agriculture (2015)
Cloves	1	–	–	0	Max Rubner Institut (2010)
Coleslaw	7	66	70	10–110	US Department of Agriculture (2015)
Coleslaw	2	800	800	600–1,000	Public Health England (2015)
Ginger	2	10	10	0–20	US Department of Agriculture (2015)
Herbs sauce	2	20	20	0–40	Max Rubner Institut (2010)
Mayonnaise	4	0	0	0–0	US Department of Agriculture (2015)
Mayonnaise	1	–	–	3,100	Public Health England (2015)
Mayonnaise	3	1,707	1,280	850–2,990	NFI (2015)
Mayonnaise	15	163	10	0–740	Max Rubner Institut (2010)
Mustard	1			10,560	US Department of Agriculture (2015)
Mustard	11	5,150	4,030	2,540–10,450	Lyczko et al. (2014)
Mustard	8	16,311	16,060	16,060–18,070	Max Rubner Institut (2010)
Mustard	15	17,410	18,200	4,340–20,790	Wendlinger et al. (2014)
Remoulade	2	1,775	1,775	1,410–2,140	NFI (2015)
Remoulade	6	235	190	0–450	Max Rubner Institut (2010)
Salad dressing	44	30	0	0–360	US Department of Agriculture (2015)
Salad dressing	3	0	0	0–0	Public Health England (2015)
Salad dressing	6	277	0	0–1,480	NFI (2015)
Salad dressing	61	155	0	0–1,190	Max Rubner Institut (2010)

Product	N	Mean	Median	Range	Reference
Sauces	43	60	0	0–1,210	US Department of Agriculture (2015)
Sauces	13	23	0	0–200	Public Health England (2015)
Sauces	288	80	20	0–1,180	Max Rubner Institut (2010)
Wasabi	1			40	US Department of Agriculture (2015)
Yeast extract	10	434	155	50–1,870	Max Rubner Institut (2010)

(a): The database of the Max Rubner Institut reports 22:1, without differentiation of n-9 and n-11 isomers or *cis* and *trans*. US Department of Agriculture database reports the total 22:1*c* content, without differentiation of n-9 and n-11 isomers. The database of Public Health England reports separately 22:1 n-9*c*+*t* and 22:1 n-11 *c*+*t*. Only data of 22:1 n-9*c*+*t* is included in the table. The database of the National Food Institute of Denmark reports data on 22:1 n-9*c*.

(b): The following are products included in the nutrient databases of the US Department of Agriculture, Public Health England, National Food Institute of Denmark and Max Rubner Institut for which no erucic acid has been reported. The cumulative number of samples included in both databases is given in brackets: agar (3); allspice (1); anise (3); asafoetida (1); baking powder (2); basil (6); bay leaf (4); bread sauce (3); burnet (2); capers (7); caraway seed (2); cardamom (2); cayenne (1); celery (13); chervil (7); chilli (4); chives (11); cinnamon (5); common rue (1); coriander (7); cumin (4); curry (1); dill (10); fennel (5); fenugreek (1); flavourings and essences (19); garlic powder (1); gelatine (1); ginger (4); glaze (2); glutamate (3); gravy (3); guacamole (1); gum (3); herbs mix (4); horseradish sauce (2); juniper (3); ketchup (4); leavening agent (1); lemon balm (5); liquorice (1); lovage (2); mace (2); marjoram (5); meat extract (6); mint sauce (4); mugwort (2); nut meg (3); onion powder (1); onion sauce (3); oregano (6); paprika (6); parsley (4); pectins (1); pepper (4); pickles mix (1); poppy seeds (2); rosemary (6); saffron (3); sage (4); salt (17); sauerkraut (1); savoty (2); soy sauce (5); spice mix (8); stock cubes (7); tamarind (4); tarragon (4); thyme (6); tomato ketchup (2); turmeric (3); tzatziki (3); vanilla (3); vegetable extracts (2); vinegar (10); yeast (13).

Table F.14: Erucic acid concentration (mg/kg)[(a)] in food products for infants and small children

Product	N	Mean	Median	Range	Reference
Baby food	207	0	0	0–20	US Department of Agriculture (2015)
Infant formula	94	0	0	0–0	US Department of Agriculture (2015)
Infant formula	3	46[(b)]	36[(b)]	17–86[(b)]	Rzehak et al. (2011)
Infant formula	2	26[(b)]	26[(b)]	0–51[(b)]	Billeaud et al. (1997)
Powdered infant milk	32	0	0	0–0	Zunin et al. (2015)

(a): The database of the US Department of Agriculture database reports the total 22:1*c* content, without differentiation of n-9 and n-11 isomers.

(b): mg/L.

Table F.15: Erucic acid concentration (mg/kg) in food for special nutritional use[(a),(b)]

Product	N	Mean	Median	Range	Reference
Rolls, gluten free	2	20	20	0–40	US Department of Agriculture (2015)
Sandwich, gluten free	3	50	40	40–70	US Department of Agriculture (2015)
Waffles, gluten free	1			20	US Department of Agriculture (2015)

(a): The database of the Max Rubner Institut reports 22:1, without differentiation of n-9 and n-11 isomers or *cis* and *trans*. US Department of Agriculture database reports the total 22:1*c* content, without differentiation of n-9 and n-11 isomers. The database of Public Health England reports separately 22:1 n-9*c*+*t* and 22:1 n-11 *c*+*t*. Only data of 22:1 n-9*c*+*t* is included in the table. The database of the National Food Institute of Denmark reports data on 22:1 n-9*c*.

(b): The following are products included in the nutrient databases of the US Department of Agriculture, Public Health England, National Food Institute of Denmark and Max Rubner Institut for which no erucic acid has been reported. The cumulative number of samples included in both databases is given in brackets: bread, gluten free (1); bakery products, diabetics (1); candies, diabetics (3); chocolate, diabetics (2); diet shake mix (3); energy drink (18); ice cream, diabetics (1); isotonic drink (2); jam, diabetics (2); nutritional supplement (5); pancakes, gluten free; pastries, diabetics (1); soyburger (1); soymilk (8); vitamin supplements (2).

Table F.16: Erucic acid concentration (mg/kg) in composite dishes[(a),(b)]

Product	N	Mean	Median	Range	Reference
Beans-based meal	9	6	0	0–50	US Department of Agriculture (2015)
Beef, cooked	475	0	0	0–30	US Department of Agriculture (2015)
Beef, cooked	166	0	0	0–0	Max Rubner Institut (2010)
Beef, fried	11	3	0	0–30	US Department of Agriculture (2015)

Product	N	Mean	Median	Range	Reference
Biscuit	9	9	0	0–40	US Department of Agriculture (2015)
Burritos	12	8	0	0–40	US Department of Agriculture (2015)
Cereal-based dishes	38	89	0	0–3,080	Max Rubner Institut (2010)
Cheese-based dishes	24	111	20	0–960	Max Rubner Institut (2010)
Chicken, cooked	150	7	0	0–130	US Department of Agriculture (2015)
Chicken, cooked	2	0	0	0–0	NFI (2015)
Chicken, cooked	51	0	0	0–0	Max Rubner Institut (2010)
Chicken, fried	77	19	0	0–240	US Department of Agriculture (2015)
Chicken, fried	3	0	0	0–0	NFI (2015)
Chicken skin, roasted	1			20,000	Public Health England (2015)
Cornish pasty	1	–	–	100	Public Health England (2015)
Egg-based dishes	74	150	20	0–2,500	Max Rubner Institut (2010)
Empanadas	1	–	–	10	US Department of Agriculture (2015)
Enchilada	3	3	0	0–10	US Department of Agriculture (2015)
Chickpea dish	1	–	–	100	Public Health England (2015)
Fruit-based dishes	148	119	0	0–3,970	Max Rubner Institut (2010)
Hamburger	14	64	25	0–260	US Department of Agriculture (2015)
Hamburger	3	167	0	0–500	Public Health England (2015)
Hamburger	7	151	0	0–1,060	NFI (2015)
Hamburger	4	45	10	10–150	Max Rubner Institut (2010)
Herring, processed	3	1,650	2,000	850–2,100	NFI (2015)
Hot dog	5	250	0	0–1,250	NFI (2015)
Hash brown	8	39	39	20–120	US Department of Agriculture (2015)
Lamb, cooked	66	0	0	0–0	US Department of Agriculture (2015)
Lamb, cooked	64	0	0	0–0	Max Rubner Institut (2010)
Lamb, fried	12	20	0	0–220	US Department of Agriculture (2015)
Lasagne	8	1	0	0–10	US Department of Agriculture (2015)
Lasagne	1	0	0	0–0	Public Health England (2015)
Legume-based dishes	8	61	0	0–170	Max Rubner Institut (2010)
Macaroni	17	5	0	0–20	US Department of Agriculture (2015)
Meatballs	2	15	15	0–30	US Department of Agriculture (2015)
Meat-based dishes	787	272	0	0–5,040	Max Rubner Institut (2010)
Milk-based dishes	45	20	10	0–240	Max Rubner Institut (2010)
Moussaka	1	–	–	100	Public Health England (2015)
Nachos	4	75	75	40–110	US Department of Agriculture (2015)
Onion rings	7	33	30	0–60	US Department of Agriculture (2015)
Pancakes	13	2	0	0–20	US Department of Agriculture (2015)
Pancakes	1			200	Public Health England (2015)
Pasta	23	3	0	0–20	US Department of Agriculture (2015)
Pasta	17	6	0	0–100	Public Health England (2015)
Pasta	144	33	0	0–770	Max Rubner Institut (2010)
Pizza	50	53	20	0–300	US Department of Agriculture (2015)
Pizza	3	100	100	100–100	Public Health England (2015)
Pizza	6	0	0	0–0	NFI (2015)
Pizza	22	21	5	0–200	Max Rubner Institut (2010)
Pork, cooked	131	1	0	0–40	US Department of Agriculture (2015)
Pork, cooked	176	0	0	0–0	Max Rubner Institut (2010)
Pork, fried	10	11	0	0–0	US Department of Agriculture (2015)
Potato puffs	2	5	5	0–10	US Department of Agriculture (2015)
Potato salad	1	–	–	140	US Department of Agriculture (2015)

Product	N	Mean	Median	Range	Reference
Potato-based dishes	156	193	10	0–3,740	Max Rubner Institut (2010)
Prepared salads	271	16	0	0–490	Max Rubner Institut (2010)
Pupusas	3	17	20	10–20	US Department of Agriculture (2015)
Quesadillas	2	15	15	10–20	US Department of Agriculture (2015)
Quiche	1	–	–	200	Public Health England (2015)
Ravioli	6	3	0	0–10	US Department of Agriculture (2015)
Rice-based dishes	79	61	0	0–3,360	Max Rubner Institut (2010)
Rice-based dishes	16	1	0	0–10	US Department of Agriculture (2015)
Rice-based dishes	4	25	0	0–100	Public Health England (2015)
Rolls	22	2	0	0–40	US Department of Agriculture (2015)
Rolls	34	233	65	10–2,300	Max Rubner Institut (2010)
Salmon, grilled	1	–	–	11,000	Public Health England (2015)
Samosas	2	500	500	0–1,000	Public Health England (2015)
Sandwiches	43	28	10	0–130	US Department of Agriculture (2015)
Sandwiches	16	102	50	0–430	NFI (2015)
Sandwiches	273	195	60	0–2,330	Max Rubner Institut (2010)
Soups	128	0	0	0–10	US Department of Agriculture (2015)
Soups	6	17	0	0–100	Public Health England (2015)
Soups	376	32	0	0–1,150	Max Rubner Institut (2010)
Spaghetti	11	5	0	0–20	US Department of Agriculture (2015)
Spaghetti	1	–	–	0	Public Health England (2015)
Spring roll	1	–	–	110	NFI (2015)
Soy-based meals	12	121	0	0–400	Max Rubner Institut (2010)
Tacos	14	11	10	0–20	US Department of Agriculture (2015)
Tamales	4	8	10	0–10	US Department of Agriculture (2015)
Turkey, cooked	51	11	0	0–50	US Department of Agriculture (2015)
Turkey, cooked	27	26	20	10–110	Max Rubner Institut (2010)
Turkey, fried	1			0	US Department of Agriculture (2015)
Veal, cooked	25	0	0	0–0	US Department of Agriculture (2015)
Veal, cooked	59	0	0	0–0	Max Rubner Institut (2010)
Veal, fried	6	0	0	0–0	US Department of Agriculture (2015)
Vegetables with curry	1	–	–	200	Public Health England (2015)
Vegetable-based dishes	472	79	10	0–5,680	Max Rubner Institut (2010)
Wraps	2	30	30	30–30	US Department of Agriculture (2015)

(a): The database of the Max Rubner Institut reports 22:1, without differentiation of n-9 and n-11 isomers or *cis* and *trans*. US Department of Agriculture database reports the total 22:1*c* content, without differentiation of n-9 and n-11 isomers. The database of Public Health England reports separately 22:1 n-9*c*+*t* and 22:1 n-11 *c*+*t*. Only data of 22:1 n-9*c*+*t* is included in the table. The database of the National Food Institute of Denmark reports data on 22:1 n-9*c*.

(b): The following are products included in the nutrient databases of the US Department of Agriculture, Public Health England, National Food Institute of Denmark and Max Rubner Institut for which no erucic acid has been reported. The cumulative number of samples included in both databases is given in brackets: arepas (1); bean burger (4); bhaji (41); bouillon 81); buble and squeak (2); cannelloni (2); casserole (9); corn fritters (1); doner kebab (4); egg rolls (3); fajita strips (1); feijoa (1); goulash (1); hummus (1); noodles (14); pakora (10); pilau (7); porridge (5); potato soup (1); risotto (4); rissoles (8); salads (14); sapotrifle (3); vegetables in oil (13); vine leaves stuffed with rice (1).

Table F.17: Erucic acid concentration (mg/kg) in snacks, desserts, and other foods[(a),(b)]

Product	N	Mean	Median	Range	Reference
Corn snack	1			2,100	Public Health England (2015)
Custard	3	0	0	0–0	US Department of Agriculture (2015)
Custard	1	–	–	0	Public Health England (2015)
Custard	16	5	5	0–10	Max Rubner Institut (2010)

Product	N	Mean	Median	Range	Reference
Ice cream	26	0	0	0–0	US Department of Agriculture (2015)
Ice cream	5	0	0	0–0	Public Health England (2015)
Ice cream	4	0	0	0–0	NFI (2015)
Ice cream	131	10	10	0–50	Max Rubner Institut (2010)
Milk-based desserts	94	46	0	0–3,190	Max Rubner Institut (2010)
Pretzels	2	0	0	0–0	US Department of Agriculture (2015)
Pretzels	5	366	50	0–880	Max Rubner Institut (2010)
Pudding	35	0	0	0–0	US Department of Agriculture (2015)
Pudding	1	–	–	0	Public Health England (2015)
Pudding	75	29	10	0–360	Max Rubner Institut (2010)
Snack bars	7	13	0	0–70	US Department of Agriculture (2015)
Snacks	83	5	0	0–100	US Department of Agriculture (2015)
Snacks	10	60	0	0–300	Public Health England (2015)
Snacks	4	93	25	0–320	Max Rubner Institut (2010)
Tortilla chips	8	4	0	0–30	US Department of Agriculture (2015)
Tortilla chips	2	100	100	0–200	Public Health England (2015)

(a): The database of the Max Rubner Institut reports 22:1, without differentiation of n-9 and n-11 isomers or *cis* and *trans*. US Department of Agriculture database reports the total 22:1*c* content, without differentiation of n-9 and n-11 isomers. The database of Public Health England reports separately 22:1 n-9*c*+*t* and 22:1 n-11 *c*+*t*. Only data of 22:1 n-9*c*+*t* is included in the table. The database of the National Food Institute of Denmark reports data on 22:1 n-9*c*.

(b): The following are products included in the nutrient databases of the US Department of Agriculture, Public Health England, National Food Institute of Denmark and Max Rubner Institut for which no erucic acid has been reported. The cumulative number of samples included in both databases is given in brackets: frozen novelties (19); popcorn (2); potato chips (2).

Appendix G – Overview of repeated dose toxicity studies in rats, pigs and monkeys

Table G.1: Repeated dose toxicity studies in rats orally exposed to HEAR oils

Duration	Age/sex/strain[(g)] of the animals (N/group)	Percentage (w/w) and type of oils/fats/lipids in the diet[(l)] or given by gavage	Dose (g EA/kg bw per day)[(h)]	Critical effects	Reference
3 days	Male Wistar rats	EA-oils[(i)]: 1 g RSO (EA 50%) per 100 g bw by gavage	5[(a),(b)]	Changes in cardiac phospholipids: appearance of 22:1 and decrease in arachidonic acid (20:4 n-6) (likely leading to metabolic dysfunction)	Maranesi et al. (1972)
4 days	Weanling males Wistar (6/group)	EA-oils[(i)]: 15% interesterified RSO (45.3% EA), 15% refined RSO (50.6% EA) or 15% crude RSO (52.1% EA)	8.2, 9.1, 9.4[(a),(b)]	Myocardium: early accumulation of lipids rich in EA	Rocquelin (1973)
4 days	Weanling males Wistar (5/group)	EA-oils:15% peanut oil (0.2% EA) or 7.5% GTE (45–49% EA) Other oils: 7.5% tribrassidate (50–54% *trans* 22:1) (+ 0–22% linoleic acid)	0.04, 4.1–4.4[(a),(b)]	Heart: increase lipids (triacylglycerol accumulation) higher than with tribrassidate Increase incorporation of EA into heart lipids compared to incorporation of brassidic acid. These differences were not due to the lower intestinal absorption of the *trans* isomer	Astorg and Compoint (1978)
3 and 7 days	Weanlings male and female Wistar/Nin and CFY (8/group)	EA-oils: 20% mustard oil (48.9% EA) Control: 20% groundnut oil (0% EA)	11.7[(a)]	Growth rate: CFY rats exhibited a better growth performance compared to Wistar rats. Feeding of a mustard oil diet cause remarkable growth retardation in CFY rats (females predominantly affected). Marginal decrease in growth rate in Wistar rats fed mustard oil Heart tissue: pale and creamy indicating fat accumulation in CFY rats fed mustard oil. No effect in Wistar rats Myocardial lipids: no alterations in triglyceride and phospholipid content and tendency towards increase cholesterol concentration in Wistar rats. Increase in triglycerides (decline after 1 week) and cholesterol and no effect on phospholipid in CFY rats	Vajreswari et al. (1990)

Duration	Age/sex/strain[(g)] of the animals (N/group)	Percentage (w/w) and type of oils/fats/lipids in the diet[(f)] or given by gavage	Dose (g EA/kg bw per day)[(h)]	Critical effects	Reference
3 or 7 days	Weanling males (Wistar) (12/group)	EA-oils: 15% tribrassidate (1.2% EA), 15% hydrogenated RSO (9.5% EA), 15% RSO (41.1% EA) or 15% GTE (42.4% EA)	0.2, 1.7, 7.4, 7.6[(a),(b)]	Heart: higher accumulation of lipids with EA. Steatosis induced after 3 days by GTE is less severe than the one induced by RSO, but is equivalent after 7 days	Rocquelin et al. (1975)
7 days	Pathogen-free 4, 12 or 32 weeks old	EA-oils: 20% RSO (29.4% EA) Other oil/fat: 20% lard + corn oil (% 22:1 not reported)	7.1[(a)]	Heart: greater fatty acids deposition in weanling rats fed RSO for 1 week than did older rats similarly treated Concentration of EA in cardiac fatty acids: 26% in 4-week-old rats, 12% in 12-week-old rats and 5% in 32-week-old rats. The oldest rats RSO for 1 week were more resistant to myocardial alterations	Beare-Rogers and Nera (1972)
7 days	Young males (15/group)	EA-oils: 20% synthesised triacylglycerols with 0.9%, 2.4%, 30.4%, 31.6%, 32.2%, 50.8% or 72.6% EA Other oils: 20% synthesised triacylglycerols with octadecenoic acid (54.8 or 71.9% 18:1) or cetoleic acid (30.7% 22:1 n-11)	0.2, 0.6, 7.3, 7.6, 7.7, 12.2, 17.4[(a)]	Heart: fat accumulation with high levels of EA and cetoleic acid. More severe lesions with EA Liver: no fat deposition	Beare-Rogers et al. (1972a)

Duration	Age/sex/strain[g] of the animals (N/group)	Percentage (w/w) and type of oils/fats/lipids in the diet[i] or given by gavage	Dose (g EA/kg bw per day)[h]	Critical effects	Reference
7 days	Males (Sprague–Dawley) (10/group)	EA-oils: • 20% corn oil (0% EA), 20% RSO mixtures (2.9% or 10.1% EA) or 20% HEAR (42.9% EA) in combination with low saturated fatty acids • In addition, 2 oils with 2.4% and 8.7% EA were tested but in combination with high saturated fatty acids	0, 0.6–0.7, 2.1–2.4, 10.3[a]	Weight gain: decrease in group receiving 10.3 g/kg bw per day Heart: signs of myocardial lipidosis in corn oil group, significant increase in lipidosis in rats receiving 2.1 g/kg bw per day + accumulation of EA in lipids, and extensive lipidosis in group receiving 10.3 g/kg bw per day + increase in cardiac triacylglycerol High level EA in cardiac triacylglycerol and free fatty acids, similar incorporation into phosphatidylserine, followed by sphingomyelin	Kramer et al. (1992)
7 days	Male Weanling (15/group)	EA-oils: 0%, 2.5%, 5%, 10%, 15% or 20% RSO (29.7% EA) or 10% or 20% canbra oil (currently referred to as LEAR; 2.9% EA) (total fat content in diet was 20%)	0, 0.3, 0.7, 0.9, 1.8, 3.6, 5.3, 7.1[a]	Heart: increase deposition total fatty acid and EA at doses of 3.6 g/kg bw per day or higher At 7.1 g/kg bw per day deposition of fat globules within myocardial cells and between the myofibrils Other changes at 7.1 g/kg bw per day: interstitial oedema, myocytolytic changes in the cardiac muscle and some focal areas of necrosis	Beare-Rogers et al. (1971)

Duration	Age/sex/strain[(g)] of the animals (N/group)	Percentage (w/w) and type of oils/fats/lipids in the diet[(i)] or given by gavage	Dose (g EA/kg bw per day)[(h)]	Critical effects	Reference
Up to 7 days	Male rats Sprague–Dawley	Oral (gavage) 15 g/kg bw ethyl erucate, EA or GTE or 0, 3.75, 7.5 and 15 g/kg bw ethyl erucate	0, 3.75, 7.5, 15[(j)]	A single oral dose of ethyl erucate of 15 g/kg bw stimulates the phagocytic activity of the RES from day 2–5 (max day 3) There is a threshold below which the phenomenon no longer occurs (not observed at 3.75 g/kg bw) The RES activity is activated after 3 days in both oral administration of EA and ethyl erucate, weather no effect was observed after administration of GTE There is a relationship between the stimulation of RES and the changes observed at cellular level in hepatic protein and nucleic acid levels	Pipy et al. (1973)
7 days	Weanling rats	EA-oils: 19% of mixture (0%, 6.1%, 12.4%, 25.9%, 50.6% EA)	0, 1.4, 2.8, 5.9, 11.5[(a)]	Heart: dose-related increase in lipid levels	Mattson and Streck (1974)
8 days	Weanling males (Wistar) (6/group)	EA-oils: 15% triolein + peanut oil (0% EA), triolein + linseed oil (0% EA), GTE + peanut oil (47.2% EA) or GTE + linseed oil (48.6% EA)	0, 0, 8.5, 8.7[(a)]	Myocardium: high increase total fatty acid and triacylglycerols in rats receiving EA. Increase levels of EA in phospholipids in rats fed EA	Rocquelin (1979)
10 days	Male Sprague–Dawley rats (15/group)	EA-diet: normal diet + 10% EA ethyl ester Other diet: Normal diet (3% total lipids)	12.0[(a)]	In the EA group, marked increase in myocardium free fatty acids and in triacylglycerols and marked differences in fatty acid pattern in triacylglycerols, free fatty acids and diglycerides, but only marginal differences in phospholipids	Marzo et al. (1997)

Duration	Age/sex/strain[g] of the animals (N/group)	Percentage (w/w) and type of oils/fats/lipids in the diet[f] or given by gavage	Dose (g EA/kg bw per day)[h]	Critical effects	Reference
10 days	Male Sprague–Dawley rats	EA-diet: normal diet + 10% EA ethyl ester; with or without carnitine i.p. injection Other diet: Normal diet (3% total lipids); with or without carnitine i.p. injection	12.0[a]	EA diet produced increases in triacylglycerols and free fatty acids but no changes in phospholipids. No effects on heart mechanical activity (heart weight, rate at which the isolated heart beat or the coronary perfusion pressure) but when pressure-volume curves were determined in the paced hearts, the pressure developed by hearts from EA treated rats was reduced, indicating that EA cause a systolic as well as a diastolic dysfunction Positive effects of propionyl-L-carnitine treatment to overcome myocardial dysfunction	Pasini et al. (1992a,b)
10 days	Weanling males (Wistar) (9/group)	EA-oils:16% lard (0% EA), 5% RSO (48.0% EA) or 16% RSO (48.0% EA) Other oils: 16% PHFO (14.3% 22:1) (total fat content in diet was 21%)	0, 2.9, 9.2[a]	Myocardium: dose-related increases in triacylglycerols and 22:1 fatty acids in RSO fed rats. Dose-related incidence and severity of lipidosis in RSO fed rats	Opstvedt et al. (1979)
10 days	Young (Sprague–Dawley) Conventional breed and SPF breed Males and females (5–10/group)	EA-oils: 20% improved RSO (2.4% EA) or 20% RSO (9.8–10.3% EA) Other oils and fats: 20% peanut oil (0.31% 22:1), 20%, margarine (0.1% 22:1), 20% margarine containing RSO (1.9–2.1% 22:1) or 20% peanut oil + RSO (1.3%, 2.1% or 10.0% 22:1)	2.9, 11.8–12.4[a]	Myocardium: lipidosis in rats receiving 12.4 g/kg bw per day (in close contact with the mitochondria). Fat droplets located in the muscle fibres. Areas of small fat droplets in rats receiving 2.9 g/kg bw per day. Fat droplets seen in some locations by electron microscopy in control and in rats receiving 22:1 at a dose ≤ 1.2 g/kg bw per day, while they were not detected by light microscopy	Engfeldt and Brunius (1975a)
10 days	Male (Wistar) (10/group)	Standard rat chow with or without 10% EA After 10 days: injection of adrenaline (5 μg/kg per min) until cardiac arrest	0, 11.4[a]	Shortening of the appearance of extrasystoles (not significant). Earlier occurrence of ventricular fibrillation, pulmonary oedema (10/10 compared to 5/10 in control group) and cardiac arrest in EA group A diet rich in EA is more arrhythmogenic The heart in EA fed rats is less efficient	Lisciani et al. (1989)

Duration	Age/sex/strain[g] of the animals (N/group)	Percentage (w/w) and type of oils/fats/lipids in the diet[(l)] or given by gavage	Dose (g EA/kg bw per day)[(h)]	Critical effects	Reference
10 days	Young (Sprague–Dawley) Conventional breed and SPF breed (germ free) (5/group)	EA-oils: 20% RSO (49.2% EA) Other oils: 20% peanut oil (1.2% 22:1)	11.8[(a)]	Myocardium: numerous lipid droplets in both germ-free and conventional rats receiving 11.8 g/kg bw per day. No fat droplets in peanut oil fed rats Fatty accumulation in the heart and muscle cells occurring in rats fed RSO was not influenced by the presence or absence of a normal intestinal flora	Engfeldt and Gustafsson (1975)
12 days	7-week-old males (Wistar) (5/group)	EA-oils: 15% EA oil (44.5% EA) other oils: 15% brassidic acid oil (*trans* 22:1 n-9), 15% elaidic acid oil (*trans* 18:1 n-9) or 15% oleic acid oil (18:1 n-9)	8.0[(a)]	Triacylglycerol accumulation higher with brassidic acid than EA	Astorg (1981)
1, 3, 7 or 14 days	Weanling males (Wistar) (15/group)	EA-oils: 25% SBO (0.4% EA) or RSO (51.9% EA)	0.12, 15.6[(a)]	Heart: Intense lipidosis with max at day 3 decreasing considerably after 14 days, high content of EA in total and neutral cardiac lipid fractions Liver: No lipidosis, only small increase in EA but appreciable increase in oleic acid content of hepatic total and neutral lipid fractions No conversion of EA to oleic acid in the heart but in the liver	Bulhak-Jachymczyk et al. (1976)
20 days	Male young (Wistar) (5/group)	EA-diet: synthetic diet + 5% EA Other diet: synthetic diet + 1.5% linoleic acid (18:2 n-6)	6.0[(a)]	Higher incorporation of EA into triacylglycerols and free fatty acids in the heart than in the liver. No incorporation of EA into phosphatidylcholine or phosphatidylethanolamine but into diphosphatidylglycerol and sphingomyelin in the heart and the liver. High level of 1-stearoyl-2-arachidonoylphosphatidylcholine in the heart after feeding EA	Yasuda et al. (1980)

Duration	Age/sex/strain[(g)] of the animals (N/group)	Percentage (w/w) and type of oils/fats/lipids in the diet[(i)] or given by gavage	Dose (g EA/kg bw per day)[(h)]	Critical effects	Reference
5, 12 or 28 days	Weanling males (Wistar) (5/group)	EA-oil: 15% EA oil (44.5% EA) Other oils: 15% brassidic acid oil (*trans* 22:1 n-9), 15% elaidic acid oil (*trans* 18:1 n-9) or 15% oleic acid oil (18:1 n-9)	8.0[(a)]	Heart: mild lipidosis (effects more severe with EA than with brassidic acid) After 5 or 12 days, accumulation of triacylglycerols in rats fed brassidic acid or EA Heart triacylglycerol content decreased with time and returned to normal values after 28 days	Astorg (1981)
23 days	Weanling rats	EA-dietary fat: 20% high oleic acid (18:1 n-9) fat (0% EA), 20% high linoleic acid (18:2 n-6) fat (0% EA), 20% high linolenic acid (18:3 n-3) fat (0% EA) or 20% high EA fat (16.8% EA)	0, 0, 0, 4.0[(a)]	Effects of all diets on the composition of myocardial tissue. ATP utilisation was reduced in the cardiac muscle in animals fed the diet high in EA	Lee and Clandinin (1986)
7 or 28 days	Weanling male Sprague–Dawley rats	EA-oil: 20% LEAR (1.1% EA) or 5% or 20% HEAR (47.7% EA) or basal diet (% 22:1 n.r.)	0.3, 2.9, 11.4[(a)]	Feeding HEAR led to liver fatty degeneration, an increase in the liver weight and a decrease in the hepatic oxidation capacity of palmitic acid (likely due to the incorporation of EA into the mitochondrial membrane interfering the fatty acyl-CoA transferring system on the membranes) Feeding LEAR did not exhibit these effects	Lishi et al. (1991)
Up to 28 days Sacrifices day 3, 7, 14 or 28	Young males and females (40/group)	EA-oils: 20% mixture of lard and corn oil (0% EA) or 20% RSO (32.9% EA) Other oils: 20% partially hydrogenated RSO (26.3% 22:1) or 20% PHHO (24.1% 22:1)	0, 7.9[(a)]	Reduced feed intake and body weight gain Heart: increase amount fatty acids, most pronounced for RSO (peaked at 1 week, declining thereafter). Fat droplets visible in the myocardium after 3, 7, 14 and 28 days. Necrosis and fibrosis after 28 days Fat accumulation more severe with RSO than with PHHO Liver: accumulation of EA, reaching a peak at 14 days	Beare-Rogers et al. (1971)

Duration	Age/sex/strain[(g)] of the animals (N/group)	Percentage (w/w) and type of oils/fats/lipids in the diet[(l)] or given by gavage	Dose (g EA/kg bw per day)[(h)]	Critical effects	Reference
7–28 days	Male Sprague–Dawley or Female Sprague–Dawley + Male Chester Beatty	EA-oils: 20% LEAR (± 1% EA) or 20% HEAR (± 31% EA)[(d)] Other oils: 20% SBO or 20% peanut oil	0.2, 7.4[(a)]	Basic physiological change in plasma lipid profiles as a result of increasing age, growth or in adaptation to the diet fed Adaptation by the liver resulting in increased chain shortening capacity for 22:1 is a consequence of prolonged feeding of oils rich in this acid and may also partially explain the regression of early myocardial lipidosis Significant physiological differences exist between male and female rats in the plasma profile of saturated and essential fatty acids Differences in plasma fatty acids stemming from sex-related physiological differences in whole body fat metabolism may form the basis of lower cardiopathological involvement for females Physiological differences in the profile of fatty acids reaching the heart occur between young growing male rats fed for 1 week and those fed for 4 weeks and between male and female rats fed identical fats for the same length of time Greater sensitivity of younger rats to cardiopathological changes. Similarly, the physiological difference between the fatty acid composition of plasma lipids from female and male rats fed identical diets may be reason for the inherent lack of lesion response by the female to RSO-induced myocardial lesions	Innis and Clandinin (1980)

Duration	Age/sex/strain[(g)] of the animals (N/group)	Percentage (w/w) and type of oils/fats/lipids in the diet[(f)] or given by gavage	Dose (g EA/kg bw per day)[(h)]	Critical effects	Reference
28 days	Male weanling (Wistar) (10/group)	EA-oils: 20% corn oil (0% EA), lard – olive oil (0% EA), HEAR (23.4 or 42.7% EA) Other oils: HEAR oils hydrogenated under various conditions (20.6%, 22.1%, 38.3% and 41.0% 22:1)	0, 0, 5.6, 10.2[(a)]	Decreased feed intake, body weight gain and liver weight with diets containing native and hydrogenated HEAR oils compared to EA-free oils. Decrease correlated with EA content Low levels of EA were incorporated in carcass fat	Craig et al. (1963)
1 day-6 weeks	8-week-old males	EA-oils: 25% RSO (45% EA) Other oil: SUN (% 22:1 n.r.)	13.5[(a)]	Heart: impaired oxidative capacity of the heart mitochondria. Sharp increase in lipid content on days 3-6 in RSO fed rats, which decreased to normal values on week 5. Increased triacylglycerols and free fatty acids (remained elevated throughout the experiment) Liver: no increase in fat cells	Houtsmuller et al. (1970)
7, 21 days or 6 weeks	Male weanling (Sprague–Dawley) (2–6/group)	EA-oils: 20% corn oil (0% EA) or 20% mustard seed oil (36.6% EA)	0, 6.6[(c)]	Increase in triacylglycerol (5-fold) and EA content in the myocardium after 1 week of feeding high EA diet, near normalisation after 3 and 6 weeks. Lipidosis does not affect the contractility of the isolated papillary muscle	Kako and Vasdev (1979)
6 weeks	Male Sprague–Dawley rats Effect of cold stress (3 weeks at 4°C) (4–25/group)	EA-oils: 20% RSO (23.6% EA) Or Chow diet	4.3[(c)]	Myocardial lipidosis and large accumulation of 20:1 and 22:1 in the hearts of rats fed RSO	Hulan et al. (1976b)
1st exp: 3, 6, 14 days and 8 weeks **2nd exp**: 3, 6 and 9 days	Female weanling (10/group) Male weanling (Wistar) (3/group)	EA-oils: 20% LEAR (1.2% EA) or 20% HEAR (34.0% EA) Other oil: 2 or 20% corn oil (% 22:1 n.r.)	**1st exp**: 0.2, 6.1[(c)] **2nd exp**: 0.3, 8.2[(a)]	**1st exp**: Marked deposition of fat in cardiac tissues during first 3–6 days of HEAR oil diet with subsequent decrease, accompanied by decrease in cardiac glycogen as long as HEAR oil was fed **2nd exp**: Decrease in cardiac glycogen is reversible if HEAR oil diet is stopped within 6 days but is not reversible after 9 days	Slinger et al. (1973)

Duration	Age/sex/strain[g] of the animals (N/group)	Percentage (w/w) and type of oils/fats/lipids in the diet[f] or given by gavage	Dose (g EA/kg bw per day)[h]	Critical effects	Reference
4 and 8 weeks	Male weanling (Sprague–Dawley) (12/group)	EA-oils: 20% SBO (0% EA), LEAR (1.5% EA) or HEAR (47.3% EA)	0, 0.3, 8.5[c]	Higher body weight gain, feed efficiency ratio, protein efficiency ratio, weight of liver, kidney and epididymal fat, and serum albumin content after LEAR oil diet as compared to HEAR oil diet, but differences concerning serum total protein, triacylglycerol, cholesterol, HDL cholesterol, haemoglobin and haematocrit. The heart, kidneys, and testes were slightly larger after SBO diet than after LEAR diet	Chun et al. (1988)
8 weeks	Male adult (Wistar) (10/group)	Diet containing 10% mixture of linoleic and linolenic acid (0% EA) or 10% EA	0, 9.0[c]	Concentration of EA was higher in fat from the heart and adrenal than from the serum, small intestine, liver and kidney	Kanazawa et al. (1984)
8 weeks	Male weanling (albino) (8/group)	EA-oils: 12% partially hydrogenated SBO (0% EA) or 12% HEAR (46.0% EA)	0, 5.0[c]	Incorporation of EA at low levels (1–2% of fatty acids) into total lipids from heart and liver mitochondria, and from liver microsomes	Tahin et al. (1981)
8 weeks	Male weanling (Wistar) (10/group)	EA-oils: 17.5% corn oil (0% EA), 17.5% LEAR (1.0% EA), 17.5% HEAR (46.0% EA) or 17.5% mustard oil (55.1% EA) each diet with 0.04 ppm or 0.43 ppm Se	0, 0.2, 7.2, 8.7[c]	Feeding of HEAR without Se led to an increase in peroxidised lipids in the serum. Increased platelet aggregation was observed after feeding of mustard oil and increased platelet ATP release after feeding of mustard oil and HEAR	Watkins et al. (1995)
8 weeks	Weanling male (Sprague–Dawley) (10/group)	EA-oils: 20% corn oil (0% EA), 20% Zephyr RSO (0.6% EA), 20% Oro RSO (1.8% EA), 20% Span RSO (4.8% EA) or 20% Echo + Arlo RSO (23.6% EA)	0, 0.1, 0.3, 0.9, 4.2[c]	Dermal lesions: after 4–5 weeks in rats receiving diets containing RSO: alopecia, scaly, haemorrhagic and necrotic tails, scaliness of the feet. Lesions became most severe at weeks 5–6 and disappeared by week 14 Increased incidence and severity of the lesions: Zephyr > Echo + Arlo > Oro ~ Span. No lesions observed in rats fed corn oil	Hulan et al. (1976a)

Duration	Age/sex/strain[(g)] of the animals (N/group)	Percentage (w/w) and type of oils/fats/lipids in the diet[(i)] or given by gavage	Dose (g EA/kg bw per day)[(h)]	Critical effects	Reference
7, 14, 28 days or 8 weeks + sacrifice day 0	Weanling male (Wistar) (6/group)	EA-oils: 15% RSO (46.3% EA) or 25% RSO (47.2% EA) Other oils: 15% SUN (1.6% 22:1), 25% SBO (2.4% 22:1) or 25% SUN (2.2% 22:1) +/− training on electric treadmill	8.3, 14.2[(a)]	Growth rate: diminution in highest EA dose group Liver weight: increased in rats fed RSO diets Myocardium: fatty degeneration in RSO rats (peak on day 7, disappearing on day 28 or 56), more pronounced in RSO 25% group than in RSO 15% group. Less intense fatty degeneration and lower content EA in trained RSO 25% rats than in untrained rats; but contrary observed in 15% RSO rats. Histiocytic granulomata observed in all rats fed RSO Skeletal muscles: absence of fatty degeneration (or negligible) in RSO fed rats Exercise decrease growth rate in all groups, influenced composition of fatty acids in myocardium and accumulation of EA, and increase the number of histiocytic granulomata	Ziemlanski et al. (1975)
3, 7, 15, 30 days or 8.6 weeks	Weanling males (Wistar) (55/group)	EA-oils: 15% refined peanut oil (0.4% EA), 15% canbra oil (currently referred to as LEAR; 0.4% EA), or 15% RSO (50.6% EA)	0.1, 0.1, 9.1[(a)]	Myocardium: early important accumulation of triacylglycerols + other fatty acids in rats fed RSO (max at 1 week) Heart steatosis disappeared progressively, but levels of EA were still non-negligible after 60 days. EA was also found in heart phospholipids as early as 3 days after feeding Myocardiac lesions in rats fed RSO (lipid infiltration 1st week, non-lipidic vacuoles appearing on day 15 and increasing thereafter, degenerative lesions of muscular fibres appears from day 15 or 30, necrosis and fibroses appears from day 30) and LEAR (degenerative lesions, necrosis and fibroses). Effects more severe with RSO than LEAR	Rocquelin et al. (1973)

Duration	Age/sex/strain[g] of the animals (N/group)	Percentage (w/w) and type of oils/fats/lipids in the diet[f] or given by gavage	Dose (g EA/kg bw per day)[h]	Critical effects	Reference
1 or 10 weeks Sacrifice at week 1 or 10	Pathogen-free (12/group)	EA-oils: 0%, 10% or 20% RSO (29.4% EA) (total fat content in diet was 20%)	0, 2.6, 5.3[c]	Heart: lower levels of cardiac fatty acids in rats fed RSO for 10 weeks than in those fed for 1 week Higher concentration of long-chain fatty acids, fat droplets and increased incidence of necrotic lesions in rats receiving 5.3 g/kg bw per day than in rats receiving 2.6 g/kg bw per day No effect on fatty acid analysis or histological staining between rats receiving 5.3 g/kg bw per day for 1 week or control group	Beare-Rogers and Nera (1972)
10 weeks	Male and female Sprague–Dawley rats 3 weeks of age 5/group housed individually (1) or in groups of 5 (2)	EA-oils: 15% Zephyr RSO (0.8% EA), 15% Span RSO (3.7% EA) or 15% HEAR (20.6% EA) Other oil: 15% SBO or 15% hydrogenated LEAR	0.1, 0.5 and 2.8[c]	Ratios of monounsaturated to saturated fatty acid: 1.6, 7.9, 17.0 and 18.2 No effect on bw gain for Zephyr oil; lower bw gain for Spann or HEAR oils compared to soybean oil Liver: no effect spleen: no effect Heart: focal lesions: degenerating muscle fibres, macrophages taking up cellular remains (more frequent in males than females). Incidence similar in different groups but severity tended to be greater in rats fed rapeseed oil diets	Vogtmann et al. (1975)
6–10 weeks 6–8 weeks	Male Wistar rats (2,5-months) Male Wistar rats (3-months)	Exp. 1: 25% RSO: 15% (or 41.2.6%) EA or 0.5% (or 15.2%) EA Exp. 2: 15% RSO:: 15% (or 41.4%) EA or 0.5% (or 15%) EA Exp. 3: 15% RSO (0.5% EA or 50.4% EA Control: sunflower oil in all exp.	3.4 and 9.3[c] 2.0 and 5.6[c] 0.1 and 6.8[c]	Adrenals: functional modifications. Amount of damages proportional to the level of EA in the diet (increase in cholesterol, decrease corticosterone	Ziemlanski et al. (1977)

Duration	Age/sex/strain[g] of the animals (N/group)	Percentage (w/w) and type of oils/fats/lipids in the diet[f] or given by gavage	Dose (g EA/kg bw per day)[h]	Critical effects	Reference
7 days, 5.7 or 11.4 weeks	(50/group)	EA-oils: peanut oil + RSO (2.2% EA in the diet) or RSO (5.4% EA in the diet) Other oil: peanut oil (< 0.2% 22:1 in the diet) or hydrogenated marine oil (2.2% 22:1 in the diet)	2.0, 4.9[c],[e]	Myocardium: fat accumulation pronounced on day 7 in rats fed hydrogenated marine oil, RSO or peanut + RSO oils, but decreased with time. In rats fed with peanut oil, only negligible fat accumulation was observed in muscle cells. Minor lesions in a few animals on day 7. Necrosis and scars observed in most animals on day 40 and 80. Most severe lesions in RSO group and weakest in peanut oil group	Olsen et al. (1978)
11.4 weeks	Young (Sprague–Dawley)SPF breed (germ free)(6-7/ group)	EA-oils: 10% Oro RSO (0.3% EA) or 10% RSO (40.1% EA)Other oils: 10% peanut oil (0.1% 22:1)	0.03, 3.6[c]	Myocardium: normal in peanut oil and Oro RSO oils fed rats; extensive histiocytic infiltration with lesions scattered over the entire heart in one male receiving 3.6 g/kg bw per day, minor areas with histiocytic infiltration and fatty accumulation in one male and 1 female receiving 3.6 g/kg bw per day, no pathological changes in three females.	Engfeldt and Gustafsson (1975)
1st exp: 28 days **2nd exp**: 12 weeks	Male weanling (albino, Charles Foster strain) (8/group)	**1st exp**: 20% peanut oil (0% EA), 20% evening primerose oil + peanut oil (0% EA), 20% evening primerose oil + mustard oil (45.8% EA) or 20% mustard oil (48.2% EA) **2nd exp**: 20% evening primerose oil + mustard oil (46.98% EA) or 20% mustard oil (48.2% EA)	**1st exp**: 0, 0, 11, 11.6[a] **2nd exp**: 8.5, 8.7[c]	Decreased feed intake, feed efficiency ratio and body weight gain with diets containing mustard oil compared to groundnut oil. No effect of mustard oil on heart triacylglycerol concentrations. GLA lowers triacylglycerol in the serum, heart and liver. GLA lowers VLDL and increases HDL cholesterol in serum (=beneficial effects)	Dasgupta and Bhattacharyya (2007)

Duration	Age/sex/strain[g] of the animals (N/group)	Percentage (w/w) and type of oils/fats/lipids in the diet[i] or given by gavage	Dose (g EA/kg bw per day)[h]	Critical effects	Reference
12 weeks	Weanling males (Wistar) (13/group)	EA-oils:, 15% LEAR (0.3% EA) or 15% HEAR (42.9% EA) Other oils: 15% SUN (% 22:1 n.r.)+/- treadmill programme	0.04, 5.8[c]	Decreased food consumption and growth rate related to physical training, mainly in rats fed RSO. Heart rates higher in trained rats fed with SUN or HEAR than with LEAR Increased liver weight in rats fed LEAR and even more in rats fed HEAR Increased incidence of myocardiac lesions in resting rats fed LEAR or HEAR. In trained rats, HEAR induces the highest incidence of lesions. Decrease rate of left ventricular pressure rise in trained rats, particularly in rats fed with SUN and HEAR	Rocquelin et al. (1982)
12 weeks	Weanling Male Wistar rats	EA-oils: 15% SUN (0% EA), 15% LEAR (0.3% EA) or 15% HEAR (42.9% EA) Subjected to a moderate physical training versus sedentary animals fed the same diets	0, 0.04, 5.8[c]	HEAR caused the highest incidence and number of heart lesions both in untrained and trained rats but in the latter a significant increase was observed. LEAR induced higher incidence of lesions than SUN in untrained but not in trained rats. HEAR and LEAR, as compared to SUN, induced marked changes in the fatty acid pattern of phospholipids in the heart. The authors concluded that these changes were mainly due to the high levels of n-9 monoenes as well as to the high ratio linolenic/linoleic acid (18:3/18:2 n-6)	Rocquelin et al. (1981)
13 weeks	Male Wistar rats 25 days old (10/group)	EA-oils: 10% RSO Janpol (2.8% EA), 10% RSO (39.5% EA) Other oil: 10% SBO (2.7% 22:1) or mixtures of different fats and oils	0.3, 3.6[c]	No effect on growth rate, liver, lungs, heart, kidneys, testis or spleen weights. Serum triacylglycerols: higher values in RSO and especially in Janpol fed rats. ATPase activity decreased in RSO and increased in Janpol fed rats (active bile secretion and excretion) Acid phosphatase: increase activity in RSO compared to Janpol fed rats Increase accumulation in RSO compared to Janpol fed rats Myocardiac lesions observed in RSO and Janpol fed ratsJanpol oil 'caused less pronounced changes in the determined indices of the biological and nutritional evaluation as compared with high EA RSO'	Ziemlanski and Budzynska-Topolowska (1978a)

Duration	Age/sex/strain[g] of the animals (N/group)	Percentage (w/w) and type of oils/fats/lipids in the diet[f] or given by gavage	Dose (g EA/kg bw per day)[h]	Critical effects	Reference
13 weeks	Weanling males and females (Sprague–Dawley) (20/sex/group)	EA-oils: 7.5% SBO (0% EA), 7.5% hydrogenated SBO (0% EA) or 7.5% RSO (44.1% EA), Other oils: 7.5% hydrogenated RSO (< 0.1% 22:1)	0, 0, 3.0[c]	No significant differences in body weight or diet-related pathology	Nolen (1981)

Duration	Age/sex/strain[g] of the animals (N/group)	Percentage (w/w) and type of oils/fats/lipids in the diet[i] or given by gavage	Dose (g EA/kg bw per day)[h]	Critical effects	Reference
1 week	Male (40/group) Sprague–Dawley rats 3 weeks of age	Low-fat diet (5% fat) for 14 weeks followed by EA-oils for 1 week: 20% corn oil (0% EA), or 20% RSO (1, 10, 20, 30, 40 or 50% EA) +/– Return to low-fat diet for 1 week	0, 0.1, 1.6, 4.1, 5.2, 7.0 and 8.8[c]	Growth depression and reduced feed intake in rats fed diets rich in EA. Growth depression effect of EA disappeared on return to low-fat diet Myocardial lipidosis: dose-related increase in severity, reversible after return to low-fat diet without EA. Relative lipidosis grading: no fat stain, very slight, slight, moderate, moderate and marked. Myocardial necrosis: dose-related increase in incidence (not reported for control, 7/30, 9/30, 17/30, 15/30, 9/30 and 16/30) not in severity (results not linear)Cardiac phospholipids: higher concentration	Kramer et al. (1988)
1 week	Castrated male Sprague–Dawley rats (40/group) Female (40/group)	Low-fat diet (5% fat) for 14 weeks followed by EA-oils for 1 week: 20% RSO (50% EA) +/- Return to low-fat diet for 1 week	8.8	Lower growth rate and feed intake than male rats Myocardial lipidosis (marked) Myocardial necrosis: lower incidence compared to males Cardiac phospholipids: lower concentration compared to males	
1 week	Sprague–Dawley rats 3 weeks of age Male Sprague–Dawley rats (40/group)	Low-fat diet (5% fat) for 14 weeks followed by EA-oils for 1 week: 20% RSO (50% EA) +/- Return to low-fat diet for 1 week	8.8	Lower growth rate and feed intake than male rats Myocardial lipidosis: lower incidence compared to males (marked) Myocardial necrosis: lower incidence compared to males Cardiac phospholipids: lower concentration compared to males	
15 weeks	3 weeks of age	Low-fat diet (5% fat) for 14 weeks followed by EA-oils for 15 weeks: 20% RSO (50% EA)	8.8	Myocardial lipidosis: severity significantly reduced compared with rats fed EA diet for 1 week (moderate), but still relatively high residual lipidosis (result from high dietary concentration of EA) Myocardial necrosis: much higher incidence and severity than after 1 week exposure to EA (incidence: 20/20, severity 18 rats with more than 6 lesions/heart)Cardiac phospholipids: higher concentration compared to males exposed for 1 week	

Duration	Age/sex/strain[g] of the animals (N/group)	Percentage (w/w) and type of oils/fats/lipids in the diet[f] or given by gavage	Dose (g EA/kg bw per day)[h]	Critical effects	Reference
1 or 16 weeks	Weanling males (COBS®)	EA-oils: 0, 2.5, 5, 10, 15 or 20% RSO (38.1% EA) Other oils: 0-15% partially hydrogenated RSO (35.2% 22:1) or 0-15% PHHO (31.3% C22:1) (total fat content in diet was 20%)	0; 0.9; 1.7; 3.4; 5.1; 6.9[c]	Heart: accumulation of lipids mainly in triacylglycerols (high concentration of 22:1). Increased fatty acids after 1 week after which it regressed. Development of degenerative lesions (necrosis and fibrosis) at week 16. Incidence of lesions in RSO rats at week 16: 0/12, 0/12, 3/12, 3/12, 6/12, 9/11. Similar incidences of lesions were observed in rats fed with partially hydrogenated RSO or partially hydrogenated herring oil.	Beare-Rogers et al. (1972b)
3-7 days or 16 weeks	3-week-old male (Sprague–Dawley) (15/group)	EA-oils: 20% SBO (0% EA), Tower RSO (0.3% EA), Tower RSO + EA (0.8% EA), SBO + EA (5.7% EA), Tower RSO + EA (5.9% EA) or HEAR (22.3% EA) Other oil: 20% olive oil (0.1% 22:1) olive oil + 4.5% EA	0, 0.1, 0.1, 1.0, 1.1, 4.0[c]	Growth rate: retardation in rats fed oil containing ≥5.7% EA. Heart: increase weight in rats fed HEAR after 3 days and 1 week. Slight triacylglycerol accumulation in rats fed oil containing 5.9% EA after 3 days or 1 week and severe in rats fed HEAR and decrease after 16 weeks. Higher free fatty acid levels in HEAR group at day 3 and 1 week and decreasing afterwards. High % EA in triacylglycerols and free fatty acids at day 3 and 1 week, declining after 16 weeks	Kramer and Hulan (1978)

Duration	Age/sex/strain[(g)] of the animals (N/group)	Percentage (w/w) and type of oils/fats/lipids in the diet[(i)] or given by gavage	Dose (g EA/kg bw per day)[(h)]	Critical effects	Reference
3-7 days or 16 weeks	Male (Sprague–Dawley) (30/group)	EA-oils: 20% corn oil (0% EA), LEAR (< 0.5% EA), HEAR (33.7% or 34.2% EA)	0, < 0.1, 8.1 or 8.2[(a)] 0, < 0.1, 6.1 or 6.2[(c)]	Growth rates: significantly less for group receiving HEAR with 33.7% EA than all other groups. Heart triacylglycerols (3-7 days): levels in LEAR group not significantly different from the corn oil group. Levels in HEAR group 7-12 times greater than all other groups. Total fatty acids in tissue phospholipids: level was the same among the groups. Fatty acid composition of tissue lipids: the same in rats fed both HEARs. Focal myocardial necrosis after 16 weeks: 0, 6, 3, 3 and 2/15 in corn oil, HEAR original, HEAR randomised, LEAR original and LEAR randomised, respectively.. Both fresh and old lesions were observed. No accumulation of lipid droplets observed in cardiac muscle fibres	Hung et al. (1977)
7 days; up to 16 weeks	Male weanling rats (Sprague–Dawley) (4/group)	EA-oils: 15% SBO (0% EA), 15% LEAR (0.1% EA) or HEAR (28.5% EA).	0, 0.02, 5.1[(a)] 0, 0.01, 3.9[(c)]	ATP synthesis decline with chronic feeding of the 15% (w/w) oil containing diets. Significantly reduced P/O ratios for groups fed HEAR for 11 days and for groups fed HEAR or LEAR for 112 days. Only long-term feeding of LEAR diet resulted in significant alterations in the efficiency of oxidative phosphorylation.	Clandinin (1978)
16 weeks	Weanling males & females (Sprague–Dawley)	EA-oils: 15% RSO (30.2% EA)Other oils: 15% SBO (0.06% 22:1), 15% hydrogenated RSO (< 0.4% 22:1)	4.1[(c)]	Slightly higher incidence of histiocytic infiltration of cardiac muscle and enlarged hearts in rats fed RSO	Nolen (1981)

Duration	Age/sex/strain[g] of the animals (N/group)	Percentage (w/w) and type of oils/fats/lipids in the diet[i] or given by gavage	Dose (g EA/kg bw per day)[h]	Critical effects	Reference
16 weeks	Mature males (Sprague–Dawley)	Diets containing 20% by weight fat **1st exp**: EA-oils: 20% lard + corn oil (0% EA) or 20% RSO (29.4% EA) **2nd exp**: EA-oils: 20% lard +corn oil (0% EA), 20% canbra oil (currently referred to as LEAR; 2.9% EA) or 20% RSO (38.1% EA) Other oils: hydrogenated RSO (33.1% 22:1), hydrogenated LEAR (2.6% 22:1)	**1st exp**: 0, 5.3[c] **2nd exp**: 0, 0.5, 6.9[c]	Heart: cardiac necrosis and fibrosis with both low and high EA RSO. Incidence of lesions: 0/17, 10/16 and 15/15 for lard + corn oil, LEAR and RSO, respectively	Beare-Rogers et al. (1974)
16 weeks	Mature males (Sprague–Dawley)	**5th exp**: EA-oils: 20% lard + corn oil (0% EA), 20% SBO (0% EA), 20% Oro RSO (1.9% EA), 20% crude Span oil (2.5% EA), 20% crude canbra oil (2.5% EA), 20% refined Canbra oil (2.5% EA), 20% refined Span oil (2.7% EA), 20% Span RSO (3.4% EA) or 20% regular RSO (23.3% EA) Other oils: 20% commercial oil (23.2% 22:1)	0, 0, 0.3, 0.5, 0.5, 0.5, 0.5, 0.6, 4.2[c]	Cardiac lesions: 7%, 15%, 37%, 56%, 55%, 37%, 58%, 59%, 73%	Beare-Rogers et al. (1974)

Duration	Age/sex/strain[g] of the animals (N/group)	Percentage (w/w) and type of oils/fats/lipids in the diet[l] or given by gavage	Dose (g EA/kg bw per day)[h]	Critical effects	Reference
16 weeks Sacrifices: day 3, week 1, 2, 4, 8 and 16	3-week-old male and female (Sprague–Dawley) (14/group)	EA-oils: 20% corn oil (0% EA), 20% SBO (0% EA), Oro RSO (1.8% EA), Span RSO (4.9% EA), HEAR (25.1% EA)[k]	0, 0, 0.32, 0.87, 4.5[c]	Myocardium: high deposition of fat with a high content of EA after 1 week in rats receiving the HEAR diet, declined to normal levels after 4 and 16 weeks. Increased incidence of necrosis and fibrosis observed in males after 16 weeks of feeding with EA diets. No effect on oxygen uptake and ATP production in intact mitochondria isolated after 1 week. Liver: less pronounced transient accumulation of fat	Kramer et al. (1973), Kramer (1973)
Up to 16 weeks Sacrifices: day 3, 7, 14, 28, 56 and 112	Weanling male and female (Sprague–Dawley) (42 or 84/group)	EA-oils: 5% lard (0% EA) or 20% corn oil (0% EA), 20% SBO (0% EA), 20% Oro RSO (1.8% EA), 20% Span RSO (4.9% EA) or 20% regular RSO (25.1% EA)[k] Or purina chow containing 1.9% lipids (0% EA)	0, 0, 0, 0.32, 0.87, 4.5[c]	Myocardium: dose-related lipidosis (peak at 3-7 days followed by regression). Focal necrosis and fibrosis in male rats after 112 days feeding with RSO regardless of the EA concentration and lower incidence or absence of lesions in females. No clear association between cardiac lipidosis and necrosis	Charlton et al. (1975)
16 weeks	Young Chester Beatty and Sprague–Dawley male (24/group)	EA-oils: 20% corn oil (0% EA), 20% LEAR (0.8% EA) or 20% HEAR (25.5% EA)	0, 0.1, 4.6[c]	Growth rate: considerably smaller for Chester Beatty rats. Inhibition of growth rate in both strains fed HEAR.Heart weights: temporary heavier in rats fed HEARTemporary accumulation of heart triacylglycerols and free fatty acids in HEAR fed rats Myocardium: marked lipidosis in both strains fed HEAR oil (weeks 1-4). Significantly lower incidence of focal necrosis and fibrosis in Chester Beatty rats than in Sprague–Dawley rats. Higher incidence in groups fed RSO in Sprague–Dawley rats. Liver: moderate to severe fat accumulation in both strains fed HEAR, more severe in Sprague–Dawley rats. Mild to moderate fat accumulation in rats fed LEAR	Kramer et al. (1979b)

Duration	Age/sex/strain[g] of the animals (N/group)	Percentage (w/w) and type of oils/fats/lipids in the diet[f] or given by gavage	Dose (g EA/kg bw per day)[h]	Critical effects	Reference
16 weeks	Male weanling (Wistar) (20/group)	EA-oil: 15% mixtures of RSO and peanut oil (15% or 30% EA) Other oils: 15% peanut oil (0.2% 22:1) or 15% PHHO (15% or 30% 22:1)	1.9, 3.7[c]	Lower growth rate, increase relative liver and kidney weights of rats fed RSO or PHHO. Heart: necrosis in rats fed RSO or PHHO; higher frequency in animals fed RSO	Astorg and Cluzan (1976)
16 weeks	Male weanling (Wistar) (5-14/group)	EA-oils: 15% EA oil (38.3 or 39.4% EA) other oils: 15% brassidic acid oil (*trans* 22:1 n-9), 15% elaidic acid oil (*trans* 18:1 n-9) or 15% oleic acid oil (18:1 n-9)	0, 5.2, 5.3[c]	Heart: necrosis only observed in EA fed groups. Hystiocytose frequent in all groups. Heart and adipose tissue: incorporation of both *cis* and *trans* 22:1 (more in triacylglycerols than into phospholipids). Percentage of brassidic acid in lipids is lower than that of EA. Liver: increase weight by the *trans*-monoenes and to a lesser extent by EA. Slight incorporation of both *cis* and *trans* 22:1	Astorg and Levillain (1977, 1979), Astorg and Compoint (1979)
2, 10, 30 days or 17 weeks	Adult females (albino) (6/group)	EA-oils: 0, 5, 10 or 15% mustard oil (47.1% EA)	0, 2.1, 4.2, 6.4[c]	Heart: Lipidosis after 2 day feeding at 10 or 15% mustard oil, mainly increase in cardiac triacylglycerols, 28% EA in total cardiac lipids, no cardiac fibrosis. Gradual decrease in lipidosis and EA content upon prolonged feeding. Lung: Lipidosis and increase in EA after 10 and 30 day feeding at 10 or 15% mustard oil (less pronounced than in heart). Increase in cholesterol esters in adrenals and ovaries, triacylglycerols in adipose tissue, and triacylglycerols and cholesterol esters in kidney. Heart has not been studied	Bhatia et al. (1979), Sharma et al. (1979)

Duration	Age/sex/strain[g] of the animals (N/group)	Percentage (w/w) and type of oils/fats/lipids in the diet[i] or given by gavage	Dose (g EA/kg bw per day)[h]	Critical effects	Reference
18 weeks	Weanling (16/group)(Wistar)	EA-oils: 20% corn oil (0% EA), Tower RSO (0.88% EA), RSO cv 1788 (3.6% EA) or Target RSO (38.9% EA)	0, 0.2, 0.6, 7.0[c]	Myocardium: –High dose group: loosening of myofibrils and slight increase in number of mitochondria (few had lost their cristae) in cardiac myocytes. –Mid dose group: large intravascular lipid droplets and some small lipid droplets in association with mitochondria. Apparent increase in number of mitochondria (normal and giant size, with distortion of shape and degeneration of cristae). –Low dose group: most pronounced degenerative changes of mitochondria, some megamitochondria showed a complete loss of cristae and a replacement of matrix with lipid-like material	Bhatnagar and Yamashiro (1979)
18 weeks	Weanling male (Wistar)(17/group)	EA-oils: 20% corn oil (0% EA), 20% Tower RSO (0.88% EA), 20% RSO cv. 1788 (3.6% EA) or 20% Target RSO (38.9% EA)	0, 0.2, 0.6 or 7.0[c]	Heart: foci of myocardial necrosis. Incidences: 8/16, 12/17, 17/17, 17/17 Presence of droplets in the small and minute blood vessel walls. Incidence of microvascular lesions: 1/16, 4/17, 8/17, 14/17, respectively	Umemura et al. (1978)
12 weeks (males) 20 weeks (females)	Male and female weanling (Wistar) (5/group)	EA-diet: diet containing 5% corn oil (0% EA) or 5% methyl erucate Other diets: containing 5% methyl 11-eicosenoate or 5% methyl oleate	0, 4.5[c]	Deposition of all fatty acids administered with the diet was observed in the body fat	Hopkins et al. (1957)
12 weeks (males) 20 weeks (females)	Male and female weanling (Wistar) (5/group)	EA-diet: diet containing 5% corn oil (0% EA) or 5% methyl erucate Other diets: containing 5% methyl 11-eicosenoate or 5% methyl oleate	0, 4.5[c]	No effect of diet on feed consumption, feed efficiency and body weight. No gross or histopathological abnormalities of the lung, heart, arteries, liver, kidney and bladder with any of the diets	Murray et al. (1958)

Duration	Age/sex/strain[g] of the animals (N/group)	Percentage (w/w) and type of oils/fats/lipids in the diet[i] or given by gavage	Dose (g EA/kg bw per day)[h]	Critical effects	Reference
Up to 20 weeks	4-week-old males and females (10/sex/group)	EA-oils: 20% peanut oil (0.2% EA) or 20% RSO (10% EA)	0.05, 2.4[a]	Heart: marked morphological changes after 8 weeks, fatty infiltration in RSO group. Absence of fatty infiltration in rats fed RSO and the rats transferred to peanut oil 5 weeks before killing. No effects on the ECG after 8 weeks Renal concentration capacity in females 20% lower than in rats receiving peanut oil on weeks 9, 10 and 20	Berglund (1975)
10, 20, 30 days or 21.4 weeks	Male albino (25/group)	EA-oils: 15% peanut oil (0% EA), RSO (42.5% EA) or mustard oil (44.2% EA)	0, 7.7, 8.0[a] 0, 5.7, 6.0[c]	Heart triacylglycerols: significant increase in mustard and RSO groups after 10 and 20 days feeding. Large amounts of EA found. Cholesterol ester: increased in mustard and RSO groups after 150 days feeding. Moderate amounts of EA found. Heart collagen content: higher in mustard and RSO fed rats after 30 and 150 days feeding	Ray et al. (1979)
Up to 23 weeks	Young (Sprague–Dawley) Males and females	EA-oils: 20% LEAR (0.3% EA) or HEAR (40.1, 46.6 or 49.2% EA) Other oils: 20% peanut oil (0.1, 0.4 or 1.2% 22:1)	11.5, 12.4[a] 0.054, 8.8, 8.8, 9.3[c]	Growth retardation in rats receiving HEAR. Myocardium: fatty accumulation (declining with time) in HEAR fed rats for 10 days. Focal myocardial lesions (histiocytic infiltration, occurrence of macrophages, myolisis, proliferation of fibroblasts and scarring) in HEAR fed rats for 40 days	Engfeldt and Brunius (1975b)
24 weeks Sacrifice week 1 and 24	3-week-old Male (Wistar) (6+12/group)	EA-oils: 30% SUN (0% EA), 25% RSO (8.5% EA) + 5% SUN, 25% HEAR (50.1% EA) + 5% SUN or 15% GTE (84% EA) + 15% SUN	0, 1.9, 11.3, 11.3[c]	Heart: lipidosis after 1 week in all groups fed with EA. Minimal in RSO group but severe in HEAR and GTE. Mononuclear cells infiltration in 2 HEAR rats. Lipidosis decreased markedly after 24 weeks, at which time fibrosis was the main pathological feature. Incidence of cellular and/or fibrotic scars: 0/12, 8/12, 12/12, 12/12, for rats receiving SUN, RSO, HEAR and GTE, respectively. Kidneys: casts and/or scars, as well as tubular dilatation were observed more frequently in HEAR and GTE fed rats	Abdellatif and Vles (1973a)

Duration	Age/sex/strain[g] of the animals (N/group)	Percentage (w/w) and type of oils/fats/lipids in the diet[f] or given by gavage	Dose (g EA/kg bw per day)[h]	Critical effects	Reference
25 weeks	Male (Wistar)	EA-oils: 20% safflower oil (0% EA), 20% SBO (0% EA), 20% hydrogenated coconut oil (0% EA), 20% simulated low EA oil (0% EA), 20% LEAR (Tower, 0.8% EA), 20% soybean high EA oil (Brassica) (26% EA), 20% soybean high EA oil (Non-Brassica) (28.5% EA), 20% low linolenic high EA oil (28.7% EA), 20% HEAR (28.8% EA) or 20% simulated high EA oil (29.9% EA)	0, 0, 0, 0, 0.14, 4.7, 5.1, 5.2, 5.2, 5.4[c]	Heart: most severe cardiac necrosis, both fresh and relatively old, in rats receiving high doses of EA (4.7-5.4 g/kg bw per day). Absence of linolenic acid from HEAR oil blend → marked reduction in incidence/severity of lesions	McCutcheon et al. (1976)
24 or 26 weeks	Males (Sprague–Dawley)	EA-oil/diet: 20% RSO (8.9% EA in the diet) or a standard diet containing 8.9% EA Other oil: SUN (% 22:1 not reported)	8.0, 8.0[c]	Growth rate: decrease in EA treated groups in first 4 weeks. Myocardium: No changes to the intrinsic myocardial contractility (*in vivo* or *in vitro*). No ECG changes. Loss of contractile reserve capacity without changes in myocardial conductance system with RSO. Interference with contractile system in peripheral vascular system with EA. In both groups, the vasoconstrictor response towards noradrenaline was profoundly reduced. According to the authors, a combination of EA and linolenic acid might be the causative factor	de Wildt and Speijers (1984)

Duration	Age/sex/strain[g] of the animals (N/group)	Percentage (w/w) and type of oils/fats/lipids in the diet[f] or given by gavage	Dose (g EA/kg bw per day)[h]	Critical effects	Reference
26 weeks	25 days old male (Wistar) (10/group)	EA-oils: 5 or 10% LEAR (Janpol, 2.8% EA), 5 or 10% HEAR (39.5% EA) Other oil: 5 or 10% SUN (1.9% 22:1),	0.1, 0.3, 1.8, 3.6[c]	Increased heart, kidney and testis weight in rats fed 10% HEAR compared to rats fed 10% SUN. Adrenal: modification of activities (levels of corticosterone) in rats fed 10% HEARLiver: no significant differences observed between groups Myocardium: lipid vacuolation, larger isolated mitochondria in rats fed 5% or 10% LEAR or 10% SUN. More alterations (increased number and size of mitochondria) were seen in rats fed 5% HEAR. No effects in rats fed 10% HEAR were reported. Serum triacylglycerols: lower levels in rats fed 10% HEAR oil ATPase activity: increase in controls and LEAR rats AP activity: higher activity in rats fed 5% HEAR oil, 10% LEAR oil or 10% SUN	Ziemlanski and Budzynska-Topolowska (1978b)
26 weeks	Young male and female (Wistar)	EA-oils: 15% peanut oil (0% EA), 15% LEAR (1.9% EA) or 15% HEAR (44.7% EA)	0, 0.3 or 6[c]	Growth rate: decrease after 6 months in males and after 2 or 3 months in females receiving HEAR Heart: weight increase in HEAR and LEAR, myocardium lesions after 6 months (90% in males for both LEAR and HEAR groups and 20% and 70% in females, for LEAR and HEAR, respectively) Liver: weight increase in HEAR and LEARKidneys: weight increase in HEAR and LEARSpleen: weight increase in HEAR and LEAR	Rocquelin and Cluzan (1968)
18 or 28 weeks	Males (Sprague–Dawley) (10/group)	EA-oils: 20% SBO (0% EA), 20% LEAR (0.9% EA) or 20% HEAR (30.9% EA)	0, 0.16 or 5.6[c]	Heart: dose-related alteration of mitochondrial morphology, disorganisation of myofibrils, degeneration or necrosis of cardiac muscle fibres in rats receiving RSO. Incidences of necrosis: 16 weeks: 0/10, 5/10 and 9/10, 28 weeks: 0/10, 6/10 and 10/10, for SBO, LEAR and HEAR, respectively	Yamashiro and Clandinin (1980)

Duration	Age/sex/strain[(g)] of the animals (N/group)	Percentage (w/w) and type of oils/fats/lipids in the diet[(f)] or given by gavage	Dose (g EA/kg bw per day)[(h)]	Critical effects	Reference
5, 7, 15, 30 days, 4 and 30 weeks	Weanling	EA-oils: 15% RSO (55% EA) Other oil: 15% peanut oil (% 22:1 n.r.)	9.9[(a)]	Heart: – first 15 days: intracellular lipidosis very pronounced. Lipid droplets are predominantly beneath mitochondria. No evident degenerative change of the myocytes. Normal mitochondrial morphology and volume. – after 2 and mainly after 7 months: Many foci of myocardial degeneration. Marked drop in the lipidosis (practically disappeared). Majority of damaged cells deprived of lipids. Increase in the average volume of mitochondria and giant mitochondria (specific to RSO). ==> None of these lesions (except effects on mitochondria) can be considered specific for RSO-induced injury. Lack of correlation between the degree of lipidosis and degenerative changes	Bodak and Hatt (1975)
30 weeks	(Sprague–Dawley) (22-26/group)	EA-oils: 4.3% or 21% peanut oil (0% EA), 10.5 or 21% HEAR (41.6% EA) Other oils: 21% PHFO (15.1% 22:1)	0, 0, 4.0, 7.8[(c)]	Heart: focal or confluent destruction of muscle cells. HEAR is significantly more cardiopathogenic than PHFO Dose-related increase in incidence and severity of lesions in rats receiving HEAR compared to the other groups. Liver: steatosis in PHFO and 21% HEARKidneys: increased % of calcified tubules in 21% HEAR and mainly in PHFO groups	Svaar and Langmark (1980)

Duration	Age/sex/strain[g] of the animals (N/group)	Percentage (w/w) and type of oils/fats/lipids in the diet[i] or given by gavage	Dose (g EA/kg bw per day)[h]	Critical effects	Reference
32 weeks Sacrifices: day 3, 6, and week 32	3-week-old (Sprague–Dawley) (24/group)	EA-oils: 0, 2.5, 5, 7.5, 10, 12.5 or 15% RSO; levels of EA in dietary fat: 0, 5.5, 11.0, 16.5, 22.0, 27.5, 33.0 (total fat content in diet was 20%)	0, 1.0, 2.0, 3.0, 4.0, 5.0, 5.9[c]	Growth rate: lower in rats receiving 5.9 g/kg bw per day Heart: dose-related lipidosis observed in all groups receiving RSO after 3 and 6 days and decreased thereafter. Fibrosis observed after 32 weeks, already in rats receiving 1.0 g/kg bw per day. Dose-related increased incidence and severity of fibrosis Mononuclear cell proliferation. Heart mild cardiopathy: 2/7, 3/8, 5/8, 2/8, 3/7, 2/8, 4/8Definite or severe cardiopathy: 0/7, 1/8, 0/8, 3/8, 3/7, 6/8, 3/8 Skeletal muscle, diaphragm: lipidosis observed after 3 and 6 days. Kidneys: slight tubular dilatation, increased debris in the lumina of renal tubules after 32 weeks (specially in group fed 15% RSO) Adrenals: lipidosis observed after 3 and 6 days. Dose-related enlargement of cortical cells from 5% RSO after 32 weeks	Abdellatif and Vles (1973a)
52 weeks	Male (Wistar)	EA-oils: 15% RSO (47.6% EA)	6.4[c]	Adrenals: excessive deposition of cholesterol Liver: change in lipid composition Heart: great deposition of EA in lipids	Ziemlanski et al. (1972a,b)
16, 32 or 64 weeks	Male newly weaned (Wistar) (12/group per sacrifice time)	EA-oils: 30% RSO (45% EA) Other oils: 30% SUN (% 22:1 n.r.)	12.2[c] 6.8[f]	Heart: foci of histiocytes and fibrosis in animals killed after 16 weeks, replacement fibrosis in animals killed at later times. Increased weight after 64 weeks in RSO group. Kidney: vacuolation of tubular epithelium, tubular dilatation and interstitial connective tissue proliferation. Increased weight after 64 weeks in RSO group. Adrenals: enlargement of cortex cells in RSO group. Pancreas: droplet vacuolation of acinar cells in RSO group. Thyroid: increased weight after 64 weeks in RSO group.	Abdellatif and Vles (1971b)

Duration	Age/sex/strain[g] of the animals (N/group)	Percentage (w/w) and type of oils/fats/lipids in the diet[i] or given by gavage	Dose (g EA/kg bw per day)[h]	Critical effects	Reference
Studies consisting of different experiments					
1 week Daily sacrifice for RSO, day 3 for control	3-week-old male (Wistar)	EA-oils: 25% RSO (45–50% EA) Other oil: 25% SUN (% 22:1 n.r.)	13.5–15.0[a]	Heart: paleness in RSO rats (already observed after day 1, increased severity after 3–6 days). Massive fatty deposition in RSO rats (increased severity after 3–6 days) Skeletal muscles: massive fatty deposition in RSO rats (increased severity with a max on day 6) No macroscopical or microscopical changes in control rats	Abdellatif and Vles (1970b)
2, 4, 8, 16, 32 or 64 weeks	3-week-old male (Wistar)	EA-oil: 30% RSO (45–50% EA) Other oil: 30% SUN (% 22:1 n.r.)	16.2–18.0[a] 12.2–13.5[c] 6.8–7.5[f]	Growth rate: slower for RSO rats Heart: pale, fatty droplets after 2 weeks in RSO rats. Decrease in severity of fatty accumulation with time. Oedema, myolysis and foci of necrosis. Focal or diffuse infiltrations of mononuclear cells, histiocytes and proliferation of fibroblasts after 4 or 8 weeks, increasing in severity thereafter. Fibrosis is the main pathological lesion observed from week 16. Increase weights. All observations described above were reported for RSO rats Adrenals: pale, droplets fatty infiltration, enlargement in RSO rats Skeletal muscles: pale after 2 and 4 weeks, but not after ≥ 8 weeks, fatty droplets after 2 weeks in RSO rats. Decrease in severity of fatty accumulation with time (complete after 16 weeks) Kidneys: some anomalies observed in RSO rats: tubular dilatation, casts and foci of scar formation from week 16 Liver: slight degree of fatty infiltration after 64 weeks in RSO rats	Abdellatif and Vles (1970b)
3–6 days	3-week-old male (Sprague–Dawley)	EA-oils: 0%, 2.5%, 5%, 7.5%, 10%, 12.5% or 15% RSO (45–50% EA) (total fat content in diet was 20%)	0, 1.4–1.5, 2.7–3.0, 4.1–4.5, 5.4–6.0, 6.8–7.5, 8.1–9.0[a]	Myocardium: fatty accumulation, severity was related to dose of RSO	Abdellatif and Vles (1970b)

Duration	Age/sex/strain[g] of the animals (N/group)	Percentage (w/w) and type of oils/fats/lipids in the diet[i] or given by gavage	Dose (g EA/kg bw per day)[h]	Critical effects	Reference
6 days	Male mature (11-week-old) (Sprague–Dawley)	EA-oils: 25% RSO (45–50% EA)	13.5–15.0[a]	Myocardium: less severe fatty accumulation than that previously found in 3-week-old Wistar rats. Extensive mononuclear cell infiltration	Abdellatif and Vles (1970b)
21 days	3-week-old male (Wistar) (12/group)	EA-oils: SBO + hardened palm oil (0% EA in the diet), RSO (8.8% EA in the diet) or GTE (8.8% EA in the diet)	0, 10.6, 10.6[a]	Growth: retardation in RSO and GTE rats Heart: lesions (similar frequency and severity in RSO and GTE) Skeletal muscles: lesions (similar frequency and severity in RSO and GTE) Absence of effects in controls	Abdellatif and Vles (1970b)
14 days	3-week-old male (Wistar) (12/group)	EA-oils: 30% SUN (0% EA), canbra oil (currently referred to as LEAR; 2% EA), RSO (49% EA)	0, 0.7, 17.6[a]	Growth & food intake: no differences between control and LEAR groups, decrease in RSO group No pathological changes in control and LEAR groups contrary to the group fed RSO	Abdellatif and Vles (1970b)
3 days	3-week-old male (Wistar)	EA-oils: 25% canbra oil (currently referred to as LEAR; 2% EA)	0.6[a]	No pathological changes observed	Abdellatif and Vles (1970b)
24 weeks	3-week-old male (Wistar) (12/group)	EA-oils: 15% SUN (0% EA), 15% canbra oil (currently referred to as LEAR; 2% EA), 15% RSO (46% EA)	0, 0.3, 6.2[c]	Growth: no differences between control and LEAR groups, decrease in RSO group Heart: high incidence mild myocardial fibrosis in RSO rats (1/12, 1/12, 9/12, respectively). Increase weights in RSO group Kidney: mild nephrosis (3/12, 4/12, 9/12, respectively), increase weights in RSO group Mild pathological changes observed in LEAR group are of the same nature and severity as those seen in the control group. They are considered as spontaneous	Abdellatif and Vles (1970b)

Duration	Age/sex/strain[(g)] of the animals (N/group)	Percentage (w/w) and type of oils/fats/lipids in the diet[(f)] or given by gavage	Dose (g EA/kg bw per day)[(h)]	Critical effects	Reference
2 weeks	3-week-old male (Wistar)	EA-oils: 30% RSO(46% EA) Other oil: 30% SUN (% 22:1 n.r.)	16.6[(a)]	Body weight gain: significantly less in RSO fed rats Heart: lighter colour, fatty infiltration, interstitial oedema and sometimes necrotic foci in RSO fed rats Adrenals: lighter colour, fatty infiltration with an enlargement of ortical cells in RSO fed rats Skeletal muscles: lighter colour, fatty infiltration (principally in red muscle fibres) sometimes with loss of striation in RSO fed rats Liver, spleen and kidneys: no pathological changes related to the diet observed in both groups	Abdellatif and Vles (1970a)
2, 4, 8 and 16 weeks	3-week-old male (Wistar) (10–12/group)	EA-oils: 30% RSO(46% EA) Other oil: 30% SUN (% 22:1 n.r.)	16.6[(a)]	Body weight gain: significantly less in RSO fed rats Heart in RSO fed rats: pale after 2 weeks, fatty infiltration that decreased after 4 and 8 weeks. Some necrotic foci, diffuse or focal proliferation of mononuclear cells, histiocytes and fibroblasts after 4–8 weeks. After 16 weeks, more fibrotic scars and histiocytic foci and disappearance of fatty infiltration Adrenals in RSO fed rats: pale after 2 weeks, fatty infiltration that slightly decreased after 4 and 8 weeks. After 16 weeks, the cells of adrenal cortex were still hypertrophied Skeletal muscles in RSO fed rats: pale after 2 weeks, fatty infiltration that decreased after 4 and 8 weeks and disappeared after 16 weeks Kidney in RSO fed rats: proteinus material and in few cases of hualine casts in the lumen of renal tubules after 16 weeks No pathological changes in the liver, spleen, testis or epidydimis	Abdellatif and Vles (1970a)

Duration	Age/sex/strain[(g)] of the animals (N/group)	Percentage (w/w) and type of oils/fats/lipids in the diet[(i)] or given by gavage	Dose (g EA/kg bw per day)[(h)]	Critical effects	Reference
2 weeks	3-week-old male (Wistar) (12/group)	EA-oils: 0%, 5%, 10%, 15%, 20%, 25% and 30% RSO (46% EA) (total fat content in diet n.r.)	0, 2.6, 5.5, 8.8, 12.6, 17.0 or 22.1[(a)]	Growth rate: slightly increased as the RSO content of the diet raised from 5% to 10% but decreased with further increases Adrenals: hypertrophy of cortical cells at ≥ 20% RSO Heart: fatty infiltration from 10% RSO (dose-related increase in severity) Skeletal muscles: fatty infiltration from 10% RSO (dose-related increase in severity)	Abdellatif and Vles (1970a)
3 weeks	3-week-old male (Wistar) (12/group)	EA-oils: 18% RSO (48% EA) or 18% GTE (48% EA)	10.4[(a)]	Pathological changes of heart, adrenals and skeletal muscles as described in exp.1 in all animals. Comparable severity between 2 groups Heart: fatty infiltration already seen after 1 day, more severe after 3–6 days Marked decrease in myocardial fat infiltration in case of discontinuation of RSO feeding after 3 days	Abdellatif and Vles (1970a)
Up to 7 days	3-week-old male (Wistar) (3/group)	EA-oil: 25% RSO(46% EA) Other oil: 25% SUN (% 22:1 n.r.)	13.8[(a)]	Heart: paleness in RSO fed rats after only 1 day, increased after 3-6 days. Fatty infiltration in all RSO fed rats on all occasions (more severe after 3–6 days) Skeletal muscles: fatty infiltration in all RSO fed rats	Abdellatif and Vles (1970a)

Duration	Age/sex/strain[(g)] of the animals (N/group)	Percentage (w/w) and type of oils/fats/lipids in the diet[(l)] or given by gavage	Dose (g EA/kg bw per day)[(h)]	Critical effects	Reference
6 days Sacrifices after 3 or 6 days	3-week-old male (Wistar) (3/group)	EA-oil: 25% RSO (46% EA) for 3 days; followed by 3 days of basal diet alone Other oil: 25% SUN (% 22:1 n.r.)	13.8[(a)]	Heart: fatty infiltration in RSO fed rats killed after 3 and 6 days (more severe after 6 days). Much less severe in groups to which RSO feeding was either discontinued or substituted by SUN	Abdellatif and Vles (1970a)

AP: alkaline phosphatase; ATP: adenosine triphosphate; bw: body weight; CoA: coenzyme A; EA: erucic acid; ECG: electrocardiogram; exp: experiment; GLA: γ-linolenic acid; GTE: glycerol trierucate; HDL: high density lipoprotein; HEAR: high erucic acid rapeseed oil; i.p.: intraperitoneal; LEAR: low erucic acid rapeseed oil; max: maximum; N: number; n.r.: not reported; PHFO: partially hydrogenated fish oil; PHHO: partially hydrogenated herring oil; P/O: phosphate oxygen ratio; RES: reticuloendothelial system; RSO: rapeseed oil; SBO: soybean oil; Se: selenium; SUN: sunflower seed oil; VLDL: very low density lipoprotein.

(a): Dose calculated using a default factor of 0.12 for a subacute study in rats (EFSA Scientific Committee, 2012).

(b): A default factor for a subacute study was used although the study was only for a few days.

(c): Dose calculated using a default factor of 0.09 for a subchronic study in rats (EFSA Scientific Committee, 2012).

(d): Percentage of erucic acid in the oil was read from a graph.

(e): To calculate the dose for the groups that received a mixture of peanut oil and rapeseed oil, it was assumed that all 22:1 present in peanut oil was erucic acid.

(f): Dose calculated using a default factor of 0.05 for a chronic study in rats (EFSA Scientific Committee, 2012).

(g): If reported by the authors.

(h): Doses are only reported for oils for which the level of EA is known.

(i): Oils for which it can be assumed that all 22:1 present is erucic acid or that do not contain 22:1.

(j): Doses reported by the authors.

(k): Weight percentage of EA calculated based on the mole% reported by the authors.

(l): When reported as cal%, the CONTAM Panel recalculated the data as weight% by using a factor of 2; to say that when reported as 22:1 it was assumed to be EA in rapeseed oil but for other oils no doses were calculated unless stated otherwise.

Table G.2: Repeated dose toxicity studies in pigs orally exposed to HEAR oils

Duration	Sex/age/species[g] of the animals (N/group)	Percentage (w/w) and type of oils/fats/lipids in the diet[i]	Dose (g EA/kg bw per day)[j]	Critical effects	Reference
10 days	Male weaned piglets (Norwegian landrace breed) (6–7/group)	EA-oil/fat: 16% lard (0% EA), 5% or 16% RSO (48.0% EA) Other oils: 20% RFO (14.6% 22:1) or 16% PHFO (14.3% 22:1)	0, 1.2, 3.9[a]	Lower bw gain in dose group receiving 3.9 g/kg bw per day Mild to moderate lipidosis in piglets receiving ≥ 1.2 g/kg bw per day	Opstvedt et al. (1979)
10 days	Male weaned piglets (Norwegian landrace breed) (7/group)	EA-oil/fat: 16% lard (0% EA), 5% RSO (48.0% EA) Other oil: 8, 12 or 16% PHFO (14.3% 22:1)	0, 1.2[a]	Mild to moderate lipidosis in piglets receiving 1.2 g/kg bw per day	Opstvedt et al. (1979)
10 days	Male weaned piglets (Norwegian landrace breed) (10 or 5/group)	EA-oil/fat: 16% lard (0% EA), 16% RSO (48.0% EA) Other oil: 12%, 14% or 16% PHFO (14.3% 22:1)	0, 3.9[a]	Heart: higher content triacylglycerols in piglets fed lard Mild to moderate lipidosis in piglets receiving 3.9 g/kg bw per day	Opstvedt et al. (1979)
14 days	Commercial baby piglets	Milk formula containing 0%, 1.75% or 3.5% RSO (29.4% EA)	0, 0.55, 0.39[f]	Heart: low levels of cardiac fatty acids in RSO fed piglets, positive fat stains in myocardium	Beare-Rogers and Nera (1972)
2–22 days; sacrifices: day 0, 2, 4, 6, 8, 10, 13, 16, 19 and 22	Male weaned piglets (Norwegian landrace breed) (3/group)	EA-oil/fat: 16% lard (0% EA) or 16% RSO (48.0% EA)	0, 3.9[a]	Heart: lipidosis detected in RSO fed piglets after 6, 8 or 13 days (maximum between 8 and 13 days and regressing thereafter)	Opstvedt et al. (1979)
Birth to 14 days	Newborn male and female Yorkshire piglets 2–20/group	EA-oils: 25% SBO (0% EA) or 25% RSO (0.8%, 2.3%, 4.7%, 7.0%, 11.7%, 20.7% or 42.9% EA) Or sow milk (0.1% EA)	0, 0.1, 0.3, 0.7, 1.1, 1.8, 3.0, 5.1[c] 0.09[b]	Heart: lipidosis in piglets fed sow milk (0.09 g/kg bw per day), disappearing by day 7. Trace myocardial lipidosis in piglets fed SBO or RSO up to 0.7 g EA/kg bw per day. Definite dose-related lipidosis in piglets receiving ≥ 1.1 mg/kg bw per day, with a maximum after 1 week diet Severity of lipidosis was higher in newborn than in weaned pigs Necrosis was not observed	Kramer et al. (1990)

Duration	Sex/age/species[g] of the animals (N/group)	Percentage (w/w) and type of oils/fats/lipids in the diet[i]	Dose (g EA/kg bw per day)[j]	Critical effects	Reference
4 weeks	Newborn Yorkshire male and female piglets (6 or 5/group)	EA-oil: 25% SBO (0% EA) or 25% of mixtures of different oils (2%, 2.2% or 20.2% EA) Or sow milk (0.1% EA)	0, 0.35, 0.39, 3.5[e] 0.02[b]	Dose-related increase in bw gain from day 9-12 and after 4 weeks a dose-related decrease in bw gain Gross or histological abnormalities: none Haematological effects: increase in platelet counts (first week) in group receiving 0.35 g/kg bw per day but thereafter the counts were significantly lower throughout the end of the study compared to the sow-reared control group. Markedly lower platelet counts throughout the 4 weeks in pigs receiving 3.5 g/kg bw per day. Increase platelet volume in group receiving 0.35 g/kg bw per day and even more in group receiving 3.5 g/kg bw per day. Decrease PCV, Hb and RBC in all groups except group receiving sow milk. Higher bleeding time in groups receiving 3.5 or 0.35 g/kg bw per day	Kramer et al. (1998)
8 weeks Sacrifices: day 0 and after 1, 2,3 and 8 weeks	Weanling male (Yorkshire) piglets (3/group)	EA-oil: 20% corn oil (0% EA) or 20% RSO (22.3% EA)	0, 2.2[a]	Heart: higher levels of neutral lipids	Kramer and Hulan (1977a)
8 weeks Sacrifices: day 0, 1, 2, 3, 4 or 8 weeks	Young male Yorkshire pigs (9–10 weeks) (3/group)	EA-oil: 20% corn oil (0% EA) or 20% refined RSO (22.3% EA)	0, 2.2[a]	Lower bw gain and food consumption in RSO group. Trace amounts of 20:1 and 22:1 fatty acids in all tissues of boars fed corn oil. Higher levels of 20:1 and 22:1 fatty acids in boars fed RSO, with a maximum within the first week in the total lipids of heart, liver and testes and remained at that level throughout the 8-week feeding trial. Significant accumulation of these long-chain monoenes was also noted in the adrenals	Kramer et al. (1975)

Duration	Sex/age/species[g] of the animals (N/group)	Percentage (w/w) and type of oils/fats/lipids in the diet[i]	Dose (g EA/kg bw per day)[j]	Critical effects	Reference
10 weeks	10- to 11-week-old Yorkshire male pigs (5/group)	EA-oil: 0% oil, 10% Tower RSO (0.4% EA) or 10% Target RSO (30.2% EA) Other oil: 10% Atlantic herring oil (% 22:1 n.r.) or 10% SBO (% 22:1 n.r.)	0, 0.02, 1.5[a]	Hepatocytes: morphological changes of endoplasmic reticulum and mitochondria in both RSO fed groups. In pigs receiving 1.5 g/kg bw per day, lipid droplets were practically absent in the parenchymal cells Bile canalicular lumina were occluded by swollen microvilli and/or globules of a lipid-like material in RSO group	Friesen and Singh (1981)
16 weeks	Male and female pigs (1st exp: 6/sex/group, 2nd exp: 16/group oils diet, 3rd exp: 2/sex/group)	**1st and 3rd exp**: EA oil: 0% oil, 15% SBO (0% EA), 15% LEAR (4.0% EA) or 15% HEAR (20.6% EA) **2nd exp**: pigs fed at about 80% of the caloric intake of exp 1	**1st and 3rd exp**: 0, 0, 0.2, 0.9[d] **2nd exp**: 0, 0, 0.1, 0.7[d]	bw gain: no significant difference between groups Histopathology: no differences on heart (focal areas of interstitial myocarditis in about 36% of all hearts), liver and spleen between different diets	Aherne et al. (1975)
16 weeks	Weanling Yorkshire male and female piglets (18/group)	EA-oils: 0% oil, 5% or 10% SBO (0% EA), 5% or 10% Oro RSO (1.6% EA), 5% or 10% Span RSO (4.3% EA) or 5% or 10% HEAR (22.3% EA)	0, 0, 0, 0.04, 0.08, 0.1, 0.2, 0.6, 1.1[a]	Growth rate: reduction in RSO groups up to week 4 Feed intake and bw gain: slightly lower for HEAR compared to lower EA diets	Friend et al. (1975)
16 weeks	Male weanling Yorkshire piglets (24/group)	EA-oils: 0% oil, 20% SBO (0% EA) or 20% RSO (22.3% EA)	0, 0, 2.2[a]	Absence of fat accumulation Heart: small foci of necrosis with infiltrations of mononuclear cells; incidence: 1/24, 1/24, 3/24, respectively	Friend et al. (1975)
24 weeks	Weaned male Yorkshire pigs (15/group)	EA-oils: 0% oil, 20% corn oil (0% EA), 20% LEAR (1.2% EA), 20% HEAR (26.3% EA)[h]	0, 0.1, 1.6[d]	Cardiac lesions: focal areas of myocardial necrosis + diffuse or focal infiltration on mononuclear cells and eosinophils in all groups, higher incidence in corn oil (73%) and HEAR (87%) compared to other groups (60%). Highest severity in HEAR group	Friend et al. (1976)
Up to 23 weeks	4–5 weeks of age piglets (8 male and 8 female/group)	EA-oils: 0% oil, 15% Tower RSO (0.3% EA), 15% LEAR (1.2% EA), 15% 1788 cv RSO (4.9% EA) or 15% HEAR (34.2% EA)	0, 0.02, 0.1, 0.4, 2.6[a]	Heart: no significant histopathological effect of RSO on incidence of cardiac lipidosis and myopathy	Aherne et al. (1976)

Duration	Sex/age/species[g] of the animals (N/group)	Percentage (w/w) and type of oils/fats/lipids in the diet[i]	Dose (g EA/kg bw per day)[j]	Critical effects	Reference
Up to 25.7 weeks (sacrifices on days 0, 6, 30, 45, 60, 90, 120 and 180)	Large white weanling male pigs (2–4/group)	EA-oils: 0% oil, 10% LEAR (2% EA) or 10% HEAR (49% EA) Other oil: 10% peanut oil (% 22:1 n.r.) or 10% SUN (% 22:1 n.r.)	0, 0.1, 2.5[a]	Myocardium: increase mitochondrial volume (due to increase in mitochondrial population and size of mitochondria) at day 30 and particularly at day 45 and day 60 in pigs fed RSO. Intramitochondrial inclusions observed in giant mitochondria. From day 60 to the end of experiment, mitochondria in different stages of degradation were observed and sometimes completely disappeared	Vodovar et al. (1977)
1, 5, 26 or 52 weeks	Female piglets (Norwegian landrace) aged 40–80 days (8/group)	EA-oils/fat: 16% lard (0% EA) or 16% RSO (42.2% EA) Other oil: 16% partially hydrogenated SBO (0.5% 22:1), 16% fish oil (18% 22:1) or 16% PHFO (17.8% 22:1)	0, 3.4[a]	Growth rate: lower for pigs fed RSO during first 6 months Heart: no macroscopic changes till week 5. Increase amount epicardial fat after 27 and 52 weeks in all groups. Slight to moderate lipidosis in pigs fed RSO or PHFO on weeks 1, 5 or 27, but not after 1 year. Minor heart lesions (muscle cell necrosis) in all groups, except in pigs fed diet containing fish oil, after 1 week and more frequently after 6 months or 1 year. No relationship between incidence and severity of heart lesions and any particular diet	Svaar et al. (1980)

bw: body weight; EA: erucic acid; PHFO: partially hydrogenated capelin oil; RSO: rapeseed oil; RFO: refined fish oil; LEAR: low erucic acid rapeseed oil; Hb: haemoglobin; HEAR: high erucic acid rapeseed oil; N: number; PCV: packed cell volume; SBO: soybean oil; RBC: red blood cell.
(a): Dose calculated assuming a body weight of 20 kg and a mean feed intake of 1,000 g/day (EFSA FEEDAP Panel, 2012).
(b): Dose from sow milk. Because of the difficulties associated with measuring milk consumption by pre-weaned piglets, few estimates are available. However, based on the studies of Theil et al. (2002) and Aguinaga et al. (2011), the CONTAM Panel assumed a milk intake of 280 g/day. From a figure in the paper, the CONTAM Panel derived a mean body weight of 3.25 kg.
(c): Mean doses calculated from the doses provided by the authors for the different ages of the animals.
(d): Dose calculated assuming a body weight of 100 kg and a mean feed intake of 3,000 g/day (EFSA FEEDAP Panel, 2012).
(e): Dose calculated based on a feed intake (dry mater) of 7% of the body weight per day as reported by the authors. It was assumed that the piglets that were fed by a sow had the same feed intake.
(f): Dose calculated using a milk intake of 280 g/day (see footnote (b) for details) and using the average body weight reported by the authors (6.95, 2.625 and 7.425 kg, respectively). Due to the low body weight for the group receiving 1.75% RSO, a higher dose than for the 3.5% RSO group was calculated.
(g): If reported by authors.
(h): Weight percentage of EA calculated based on the mole% reported by the authors.
(i): When reported as cal%, the CONTAM Panel recalculated the data as weight% by using a factor of 2; to say that when reported as 22:1 it was assumed to be EA in rapeseed oil but for other oils no doses were calculated unless stated otherwise.
(j): Doses are only reported for oils for which the level of EA is known.

Table G.3: Repeated oral dose toxicity studies in monkeys

Duration	Sex/age/strain[c] of the animals (N/group)	Percentage (w/w) and type of oils/fats/lipids in the diet[d]	Dose (g EA/kg bw per day)[e]	Critical effects	Reference
1 or 10 weeks	Squirrel monkeys (*Saimiri sciureus*) (3/group per duration)	EA-oil[b]: 0%, 10% or 20% RSO (29.4% EA)	0,1.5, 2.9[a]	Myocardium: fat globules observed after 1 week in all groups, decrease at week 10. Increase level of EA in 20% RSO fed monkeys after 10 weeks Death incidence: 2/6, 0/6, 1/6, respectively	Beare-Rogers and Nera (1972)
17 weeks	Cynomolgus monkeys (*Macaca fascicularis*) (3–4/group)	EA-oil: 25% RSO (25% EA) Other oil/fat: 25% LCO (% 22:1 n.r.) or LCO), 25% PHHO (24% 22:1)	3.1[a]	Growth rate: no effect Serum triacylglycerol: similar concentrations in 3 groups SGOT: increase activity in RSO and PHHO groups Creatine phosphokinase activity: increase in RSO group (certain time points) Heart total lipids: < 1% EA in LCO, 22% in RSO group Heart triacylglycerols: < 1% EA, 34% in RSO group Myocardium: pronounced lipidosis (lipid globules) and increased size of mitochondria in all RSO and PHHO monkeys. Foci of mononuclear cell infiltration (infrequent) in 3 groups (non-specific). Small foci of histiocytic infiltration in 2/4 monkeys of LCO and RSO groups and in 2/3 PHHO monkeys; No evidence of scarring. Mild mitochondrial degeneration cause a depression of the P/O[f] ratio of the RSO group and a State II respiratory rate depression of the PHHO group Skeletal muscles: lipidosis (lipid globules) in all RSO and PHHO monkeys. No inflammatory changes Blood pressures, contraction strength, electrical activity: no statistically significant intergroup differences	Schiefer et al. (1978), Loew et al. (1978)
17–22 weeks	Cynomolgus monkeys (*Macaca fascicularis*) (3–4/group)	EA-oil: 25% RSO (25% EA) Other oil/fat: 25% LCO (0.1% 22:1 n.r.) or LCO), 25% PHHO (21% 22:1)	3.1[a]	Growth: no adverse effect Myocardium: severe lipidosis in RSO and PHHO group. Few mild foci of inflammation in animals of all groups. Absence of mononuclear cell infiltration Skeletal muscles: severe lipidosis in RSO and PHHO groups	Ackman and Loew (1977), Ackman et al. (1977)

Duration	Sex/age/strain[c] of the animals (N/group)	Percentage (w/w) and type of oils/fats/lipids in the diet[d]	Dose (g EA/kg bw per day)[e]	Critical effects	Reference
52 weeks	Adult male Bonnet monkeys (*Macaca radiata*) (8/group)	EA-oil: 20% mustard oil (40–44% EA) other oil: 20% peanut oil (% 22:1 n.r.) or 20% hydrogenated peanut oil (% 22:1 n.r.)	4-4.4[a]	Myocardium: sarcoplasmic vacuolation of right and left ventricular + myocardial fibrosis in mustard oil fed monkeys	Gopalan et al. (1974)

bw: body weight; EA: erucic acid; LCO: lard and corn oil; n.r.: not reported; PHHO: partially hydrogenated herring oil; RSO: rapeseed oil; SBO: soybean oil; SGOT: Serum glutamic oxaloacetic transaminase.
(a): Dose calculated based on a default body weight of 5 kg and feed intake of 250 g/day (WHO, 1987).
(b): Oils for which it can be assumed that all 22:1 present is erucic acid or that do not contain 22:1.
(c): If reported by authors.
(d): When reported as cal%, the CONTAM Panel recalculated the data as weight% by using a factor of 2; to say that when reported as 22:1 it was assumed to be EA in rapeseed oil but for other oils no doses were calculated unless stated otherwise.
(e): Doses are only reported for oils for which the level of EA is known.
(f): Number of molecules of phosphorous taken up per atom oxygen.

Table G.4: Repeated oral dose toxicity studies in mice, rabbits and gerbils

Duration	Sex/age/strain[(c)] of the animals (N/group)	Percentage (w/w) and type of oils/fats/lipids in the diet[(d)]	Dose (g EA/kg bw per day)[(e)]	Critical effects	Reference
5 weeks	Male and female mice (Kunming)	EA oils: 2% RSO (4.7% or 32.6% EA)	0.2 and 1.3[(a)]	Lower levels of serum total cholesterol and triacylglycerol and slightly higher level of HDL cholesterol in mice fed LEAR oil compared to mice fed HEAR oil Heart: significant mass increases in female fed HEAR oil compared to mice fed LEAR oil and control mice. Rising trends in male mice fed HEAR oil Liver: significant mass increases in mice fed HEAR oil No effect on other visceral organs: spleen, kidney, ovary and testis	Lei et al. (2010)
84 weeks	Male 15-week-old rabbits (Viennese x Alaska) (12/group)	3 weeks: SBO then EA oil: 20% RSO (45% EA) Other oil: 20% sunflower seed oil	2.7[(b)]	BW gain: significantly less in RSO fed rabbits Heart: pale myocardium, diffuse interstitial fibrosis associated in some animals with vacuolar changes of the myocardium and disarrangement and dissociation of the myofibrils in RSO fed animals Liver: cirrhotic (portal fibrosis) associated with hyperplasia of the bile ducts and/or ductural cells in RSO animals Thoracic aorta: atherosclerotic changes in 2 RSO fed rabbits Total serum lipids (cholesterol and phospholipids) higher in RSO fed rabbits	Abdellatif and Vles (1971b)
1 week	Adult male and female gerbils (*Meriones unguiculatus*) Weanling male gerbils	EA oils: 10% RSO (2.3% or 29.4% EA) Other diets: LCO EA oil: 10% RSO (2.3% or 29.4% EA)	–[(f)]	Heart: more deposits of cardiac fatty acids including EA in HEAR oil fed gerbils, particularly in young gerbils	Beare-Rogers and Nera (1972)
1 week	Weanling gerbils	EA oils: 20% RSO (38.1% EA	–[(f)]	Heart: increase in cardiac fatty acids (peak at day 4)	Beare-Rogers et al. (1972b)

bw: body weight; EA: erucic acid; LCO: lard and corn oil; n.r.: not reported; PHHO: partially hydrogenated herring oil; RSO: rapeseed oil; SBO: soybean oil; SGOT: serum glutamic oxaloacetic transaminase.
(a): Dose EA calculated using the default factor of 0.2 (EFSA Scientific Committee, 2012).
(b): Dose EA calculated using a default feed intake of 60 g and a body weight of 2 kg (WHO, 1987).
(c): If reported by authors.
(d): When reported as cal%, the CONTAM Panel recalculated the data as weight% by using a factor of 2; to say that when reported as 22:1 it was assumed to be EA in rapeseed oil but for other oils no doses were calculated unless stated otherwise.
(e): Doses are only reported for oils for which the level of EA is known.
(f): In the absence of default values for body weight and feed intake of gerbils, the doses of erucic acid for gerbils were not estimated.